الملخص

التغيرات الموسمية في تركيز المعادن النادرة وأنزيمات مضادات الأكسدة بدم أغنام العواسي التي ترعى بجنوب الأردن

محمد أحمد محمد المطارنة

جامعة مؤتة، 2005

تمت هذه الدراسة بغرض التعرف على التغير في تركيز المعادن النادرة ونشاطية إنزيمات مضادات الأكسدة بدماء أغنام العواسي المرباة على المراعي بجنوب الأردن مع تغير الموسم والموقع. خمس وأربعون حيوان (أغنام عواسي) اختيرت من ثلاث مواقع في منطقة الكرك (عي, الجديدة والقصر). عينات الدم جمعت خلال عام كامل, إضافة إلى عينات علاف وتربة. حللت عينات الدم لتقدير تركيز النحاس(Cu), الزنك (Zn), الكوبالت (Co) والمنغنيز (Mn), ونشاطية إنزيمات الجلوتاثيون بيروكسيديز (Gpx), الجلوتاثيون المختزل (GR), الجلوتاثيون الناقل الكبريتي (GST) والكاتاليز (CAT). دلت النتائج في عي على وجود تغيرات معنوية ($P<0.05$) في تركيز Mn ,Zn وCo من موسم لآخر, لكن لم يوجد تغير معنوي ($P>0.05$) في تركيز Cu. بالإضافة وجد فروق معنوية ($P<0.01$) في نشاطية (GR) GST وCAT . في الجديدة, وجد فروق معنوية ($P<0.01$) في تركيز Znو Mn من موسم لآخر, لكن لم يكن فرق معنوي ($P>0.05$) في تركيزCu. بالإضافة كان هناك تغيرات معنوية($P<0.05$) في نشاطيةGpx, GR و GST, لكن لم يكن هناك تغير معنوي ($P>0.05$) في نشاطيةCAT. في القصر, وجد تغيرات معنوية ($P<0.01$) في تركيزCu , Zn وMn من موسم لآخر, لكن لم يكن تغير معنوي ($P>0.05$) في تركيزCo. إضافة لذلك وجد تغيرات معنوية ($P<0.05$) في نشاطية Gpx , GR و GST من موسم لآخر.

في الخلاصة, كل المواقع تعاني من نقص في تركيز المنغنيز والكوبالات طوال
العام, كما كان هناك تذبذب في نشاطية جميع الأنزيمات من موسم لآخر.

Abstract

Seasonal variation of the trace element levels and enzyme activities of grazing Awassi sheep at southern Jordan

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The study was conducted to monitor the seasonal changes in trace elements and enzyme activities in grazing Awassi ewes in southern Jordan. Forty five animals were selected from 3 locations around Al-Karak region; the locations were Aiy, Al-Jedydeh and Al-Qaser. Blood sample were divided in 4 season according to traditional feed calendar, season I (spring), II (summer), III (autumn) and IV (winter). Feed and soil samples were collected at the same time of bleeding. The effect of season and location were statistically analyzed by ANOVA. The results indicated that in Aiy a significant changes in Zn ($P<0.05$), Mn and Co concentrations, but no change in Cu concentration. Moreover, a significant ($P<0.01$) changes by season in GR activities, GST and CAT, but no change in Gpx activity by season. In Al-Jedydeh, the results indicated significant changes ($P<0.01$) in Cu concentration, Zn and Mn by season, but no change in Co concentration. Moreover, a significant changes ($P<0.05$) in Gpx, GR and GST activities, but no change in CAT activity. In Al-Qaser, a significant changes ($P<0.01$) in Cu, Zn and Mn concentrations by season, but no change in Co concentration. Moreover, a significant changes ($P<0.05$) in Gpx, GR, GST and CAT activities by season. In addition, results indicated a significant correlation ($P<0.001$) between Mn with GR and GST activities. A significant correlation ($P<0.001$) between GR and GST, Gpx and CAT activities.

In conclusion, all locations are suffered from Mn and Co deficiency throughout the year. Moreover, a fluctuation in Gpx, GR, GST and CAT activities are shown to be season dependent.

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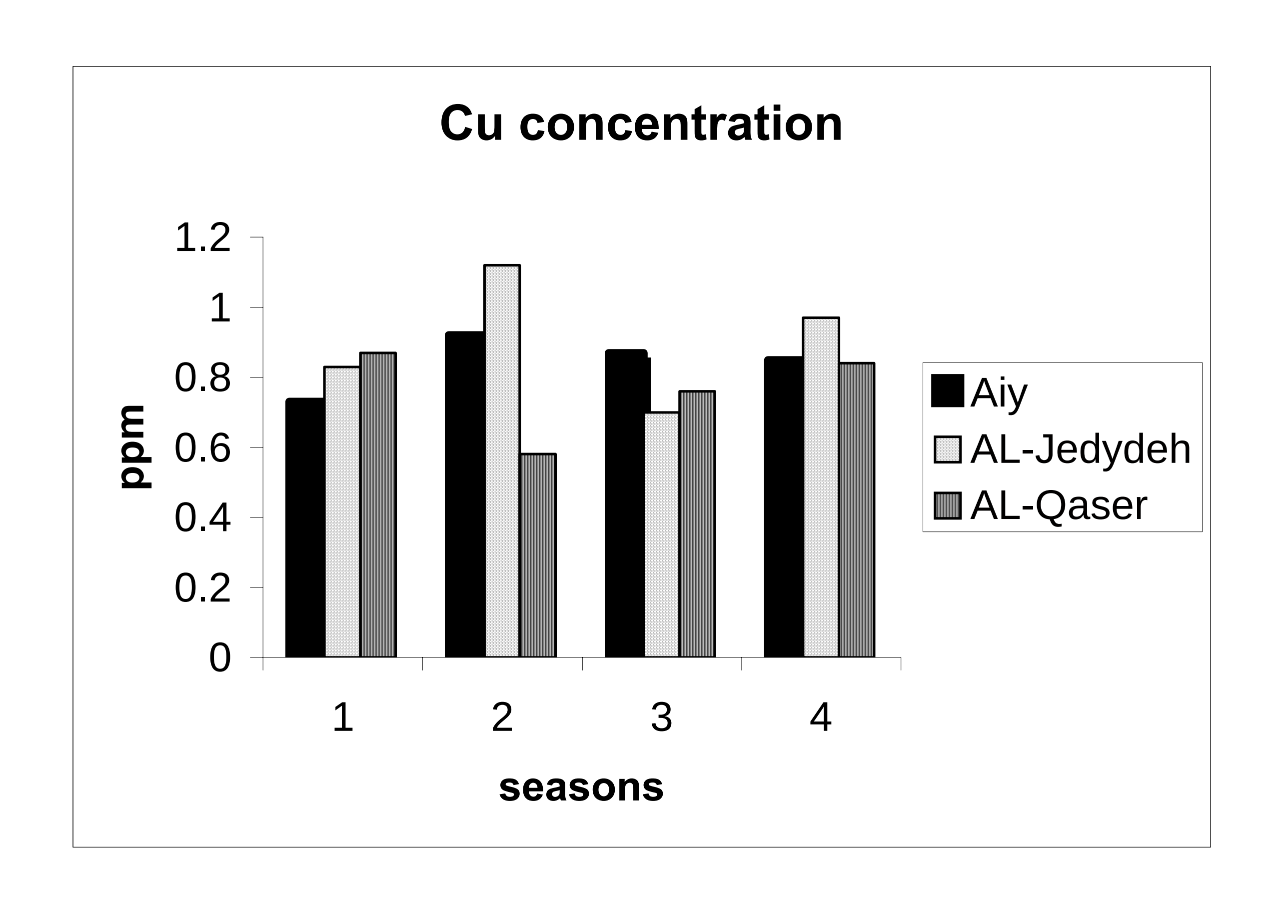


Figure 1.

Concentrations of Cu in serum of grazing Awassi sheep throughout the year in Aiy, Al-Jedydeh and Al-Qaser

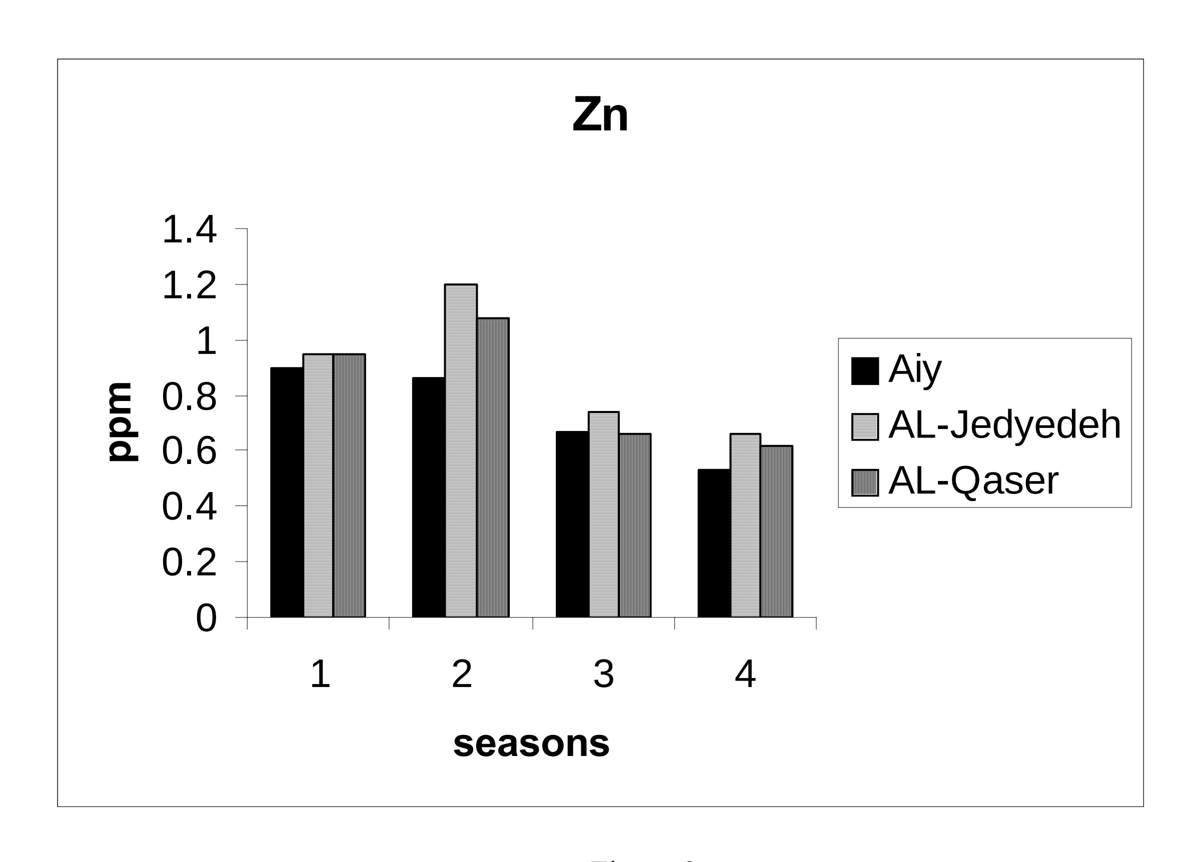


Figure 2.

Concentrations of Zn in serum of grazing Awassi sheep throughout the year in Aiy, Al-Jedydeh and Al-Qaser

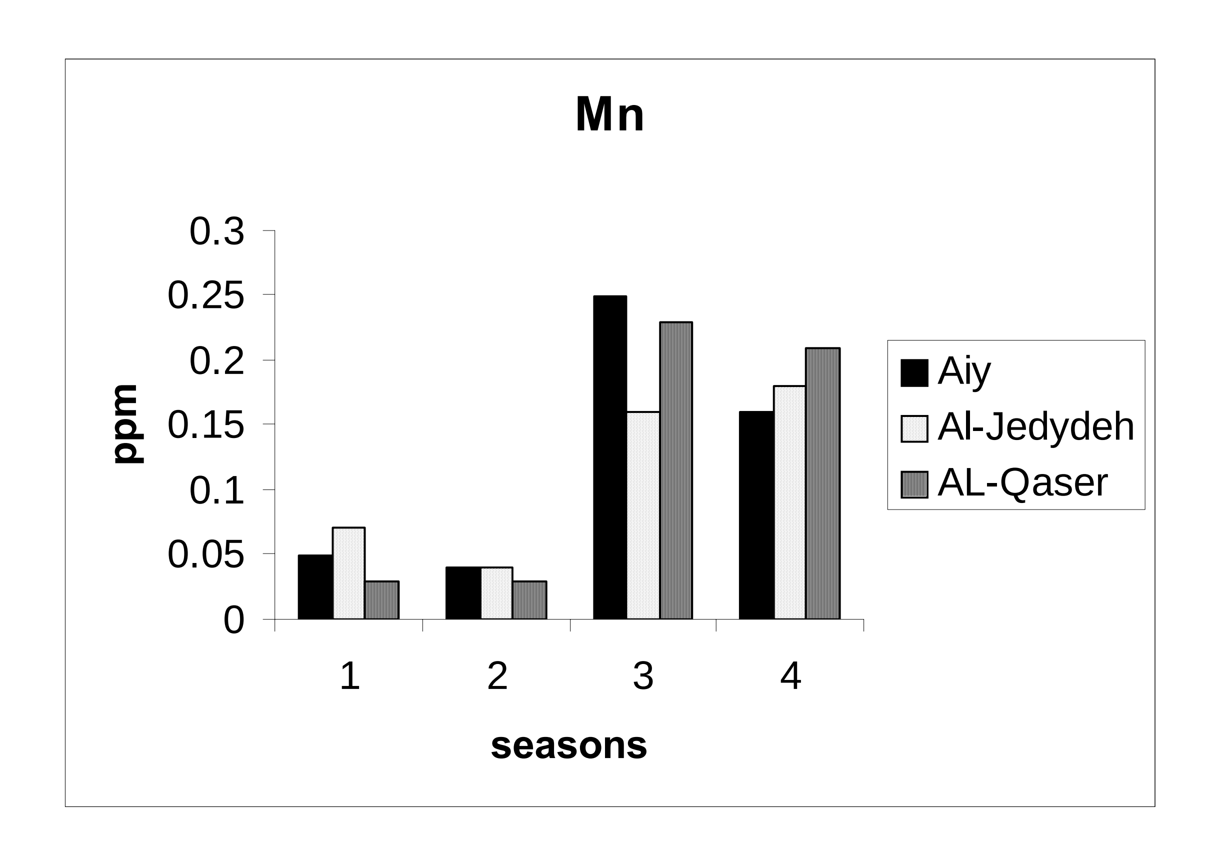


Figure 3.

Concentrations of Mn in serum of grazing Awassi sheep throughout the year in Aiy, Al-Jedydeh and Al-Qaser

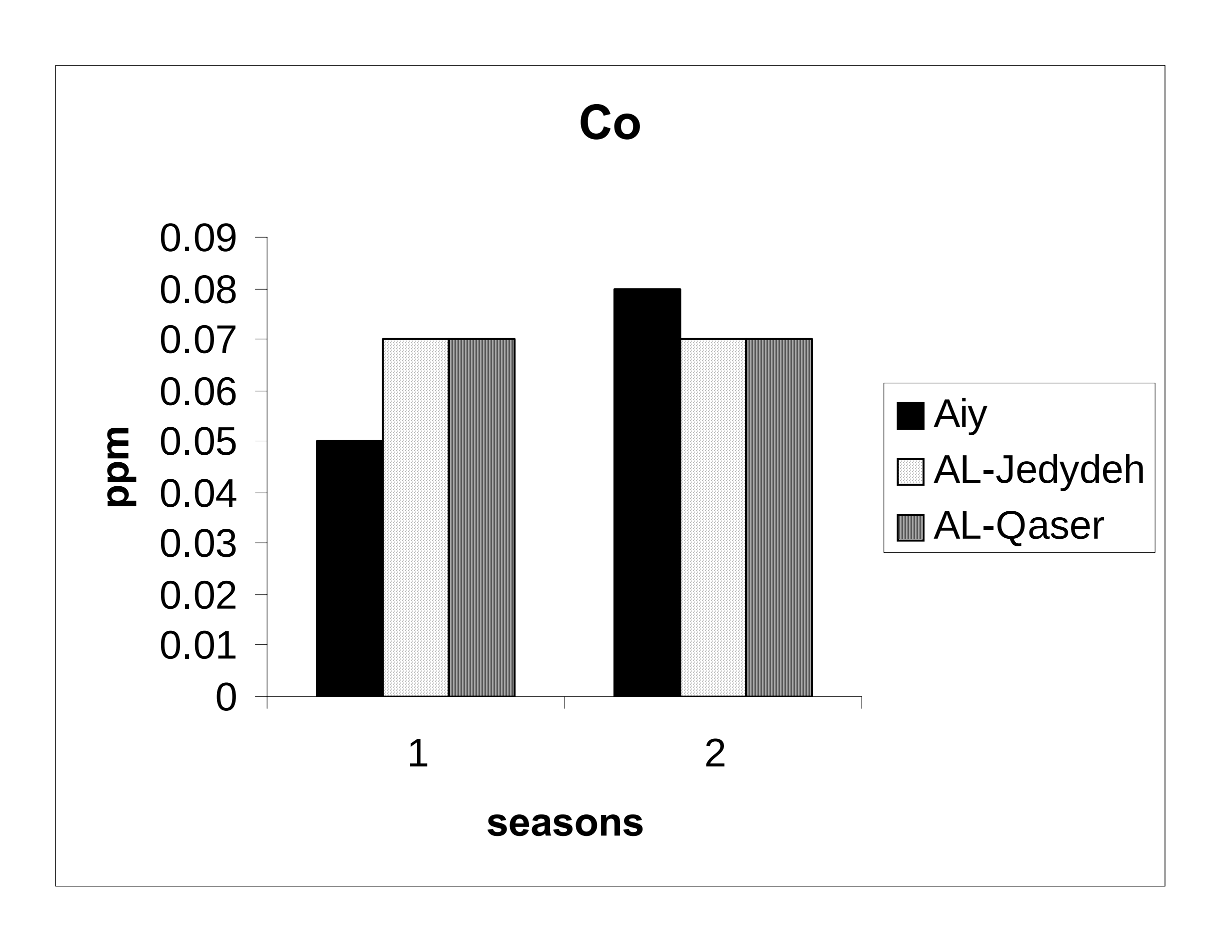


Figure 4.

Concentrations of Co in serum of grazing Awassi sheep throughout the year in Aiy, Al-Jedydeh and Al-Qaser

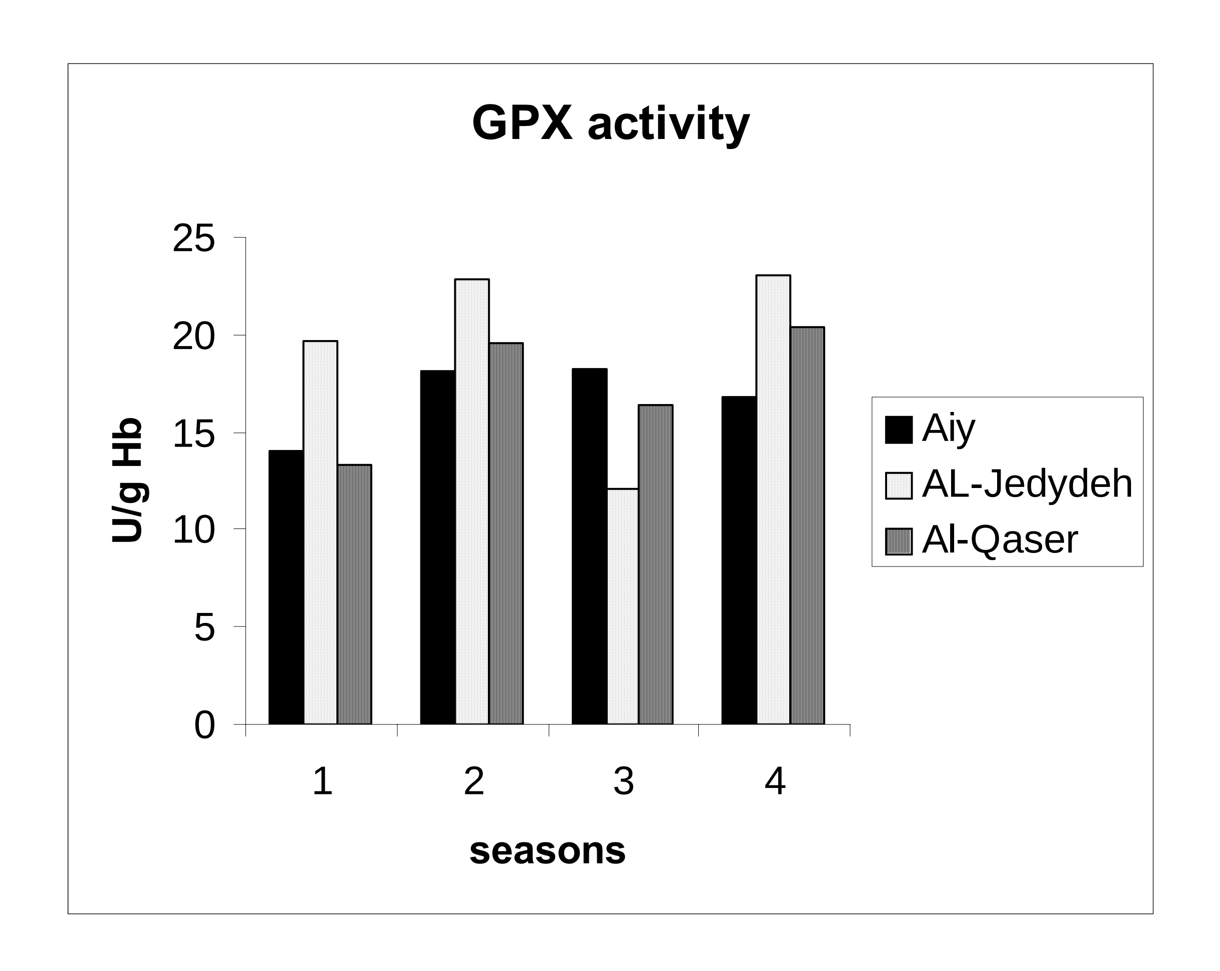


Figure 5.

Activities of Gpx enzyme in whole blood of grazing Awassi sheep throughout the year in Aiy, Al-Jedydeh and Al-Qaser

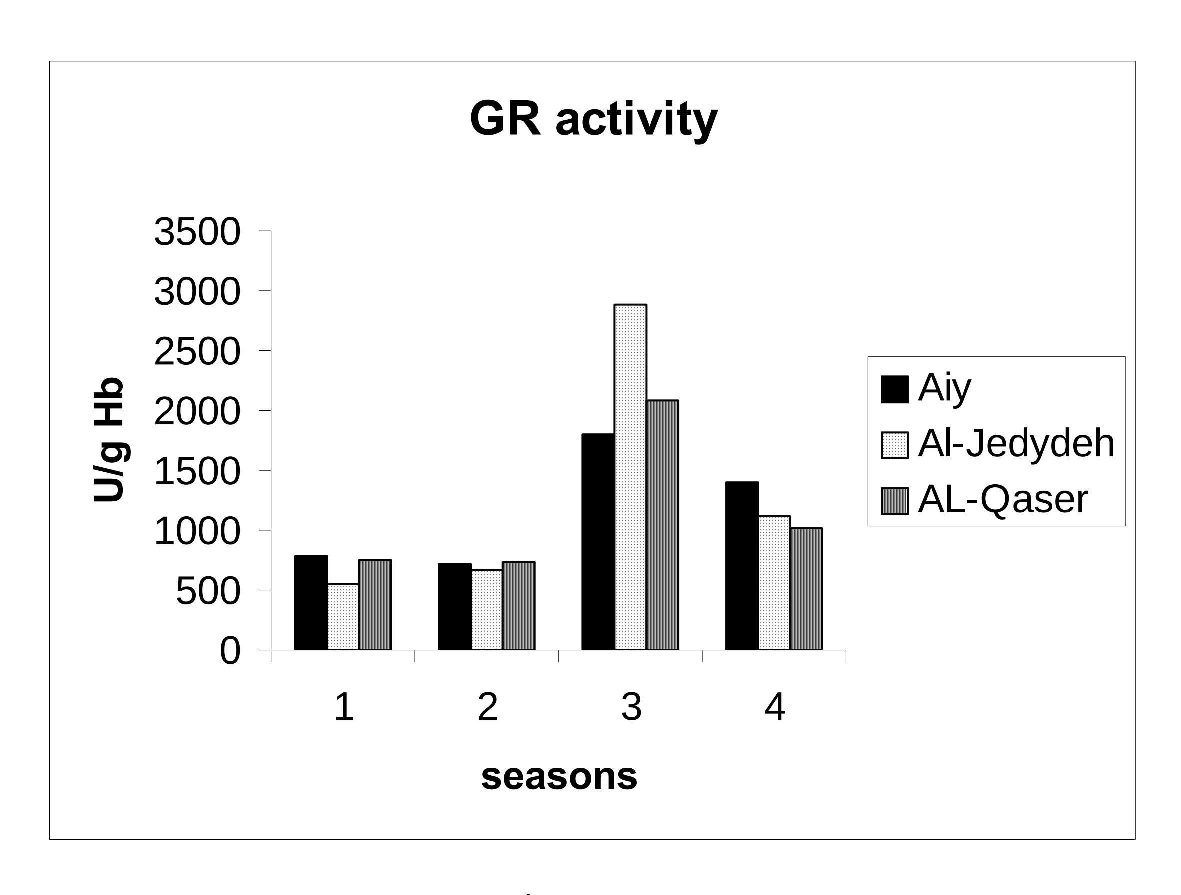


Figure 6.

Activities of GR enzyme in whole blood of grazing Awassi sheep throughout the year in Aiy, Al-Jedydeh and Al-Qaser

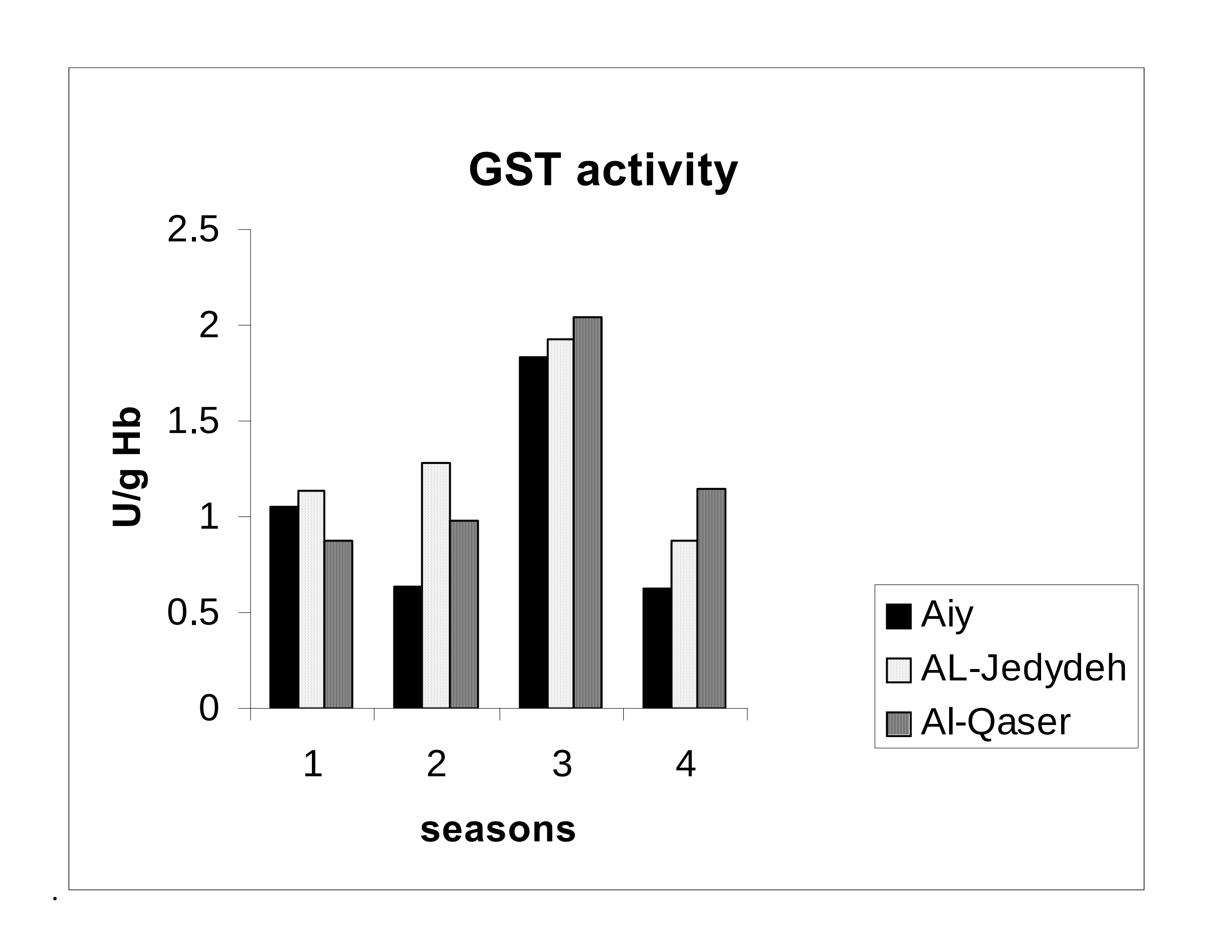


Figure 7.

Activities of GST enzyme in whole blood of grazing Awassi sheep throughout the year in Aiy, Al-Jedydeh and Al-Qaser

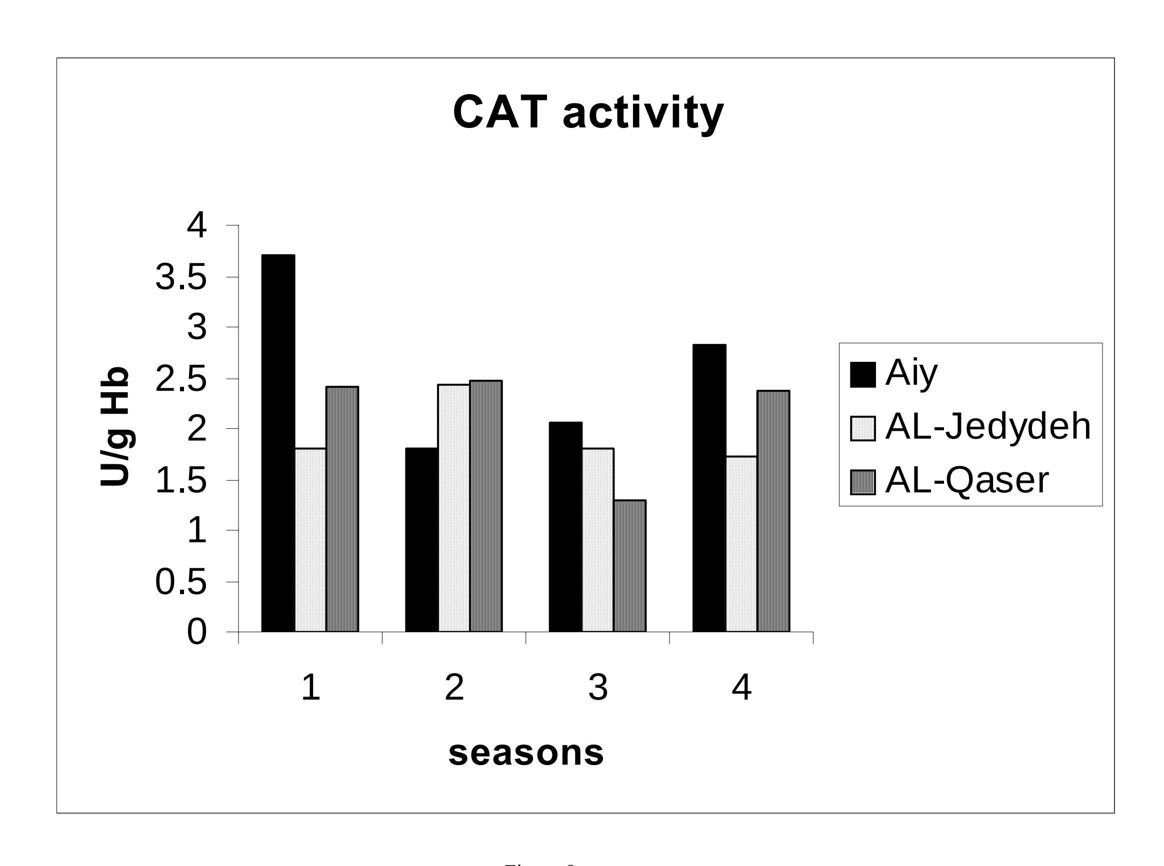


Figure 8.

Activities of CAT enzyme in whole blood of grazing Awassi sheep throughout the year in Aiy, Al-Jedydeh and Al-Qaser

Appendix (II)

1. Instruments:

1. Spectronic GENESYS 2(Milton Roy company, U.S.A.)
2. Atomic Absorption Flame and Hydride generator (Varian. GTA 100 Spectra AA- 200).
3. Gs-6 Centrifuge. (Beckman, U.S.A).
4. Laboratory centrifuge GmbH. (sigma, Germany).
5. Microprocessor PH meter. (WTW, Germany).
6. Electronic pipetting center. (HTL, High tech lab).
7. Balance. (OHAUS, U.S.A.).

2. Equipments:

1. Vaccutte tubes (hebraized and non- hebraized).
2. K_2HPO_4 and $KH_2PO_{4.}$, (Sigma- Aldrich chemi).
3. Trichloroaceticacid (1 kg). (Janssen chimica).
4. Hydrogen peroxide 30% (H_2O_2). (GAINLAND Chemical company, U.K.)
5. 1-chloro-2, 4-dinitrobenzene ($C_3H_3CIN_2O_4$) (MERCK-Schuchardt, Germany).
6. Tris- (hydroxymethyl)-amino methane (1 kg). (Reidel- de Haen AG, Germany).
7. Sodium Chloride (NaCl) (5 kg). (Sigma-Aldrich company, Germany).
8. Ethylene diamine tetra acetic acid (EDITA) disodium salt. LR. ($C_{10}H_{12}N_4Na_2O_8.2H_2O$). (S.D. Fine-chem. Ltd)
9. Hydrochloric acid (HCl) (SURECHEM).
10. Absolute Alcohol A.R. (Ethyl Alcohol C_2H_5OH). (HAYMAN, England.
11. Glutathione. (25 gm). Reduced form Minimum 98% (Sigma-Aldrich, Germany).
12. L-Glutathione oxidized (5gm). Min 98% (Applichem, Germany).
13. Glutathione Reductase. From wheat germ (1 gm). (Sigma- Aldrich, Germany).
14. NADPH-Tetra salt (500 mg). (Applichem, Germany).
15. Hemoglobine Reagent Set (6×1L Hemoglobin reagent), (1×30 ml Hemoglobin standard). (Techo Diagnostics, U.S.A.).

Chapter One
Theoretical Background

1.1. Introduction

The total number of awassi sheep in Jordan was about 1,433,310 heads. Five percent of them was in Al-Karak region (D.O.S. 2002). Grazing sheep in Jordan depend exclusively upon pasture, crop residues and grain, since they are raised as an adjunct to crop production area, that become main source of nutrient requirements (FAO, 1994).

Obviously, their productivity is below the average due to mal nutrition and health problem. Pasture is affected by season in regards of their nutritive value because of the environmental factors. The seasonal changes, short rainy season and long dry season affect the pasture quality and quantity. One of the most important nutrients is a mineral. Several trace element, such as Iron (Fe), Copper (Cu), Zinc (Zn), Selenium (Se), Manganese (Mn), Cobalt (Co), are considered to be essential for animal health. More than 90% of the body iron is presnt combination with proteins, particually haemoglobin (Mc Donald *et al*., 1987).

The assessment of trace element status of ruminants is done to determine the presence or prevalence of nutrient deficiencies (or toxicities) within a population. Assessment also done to evaluate efficacy of dietary supplementation or to compare available supplements (Kincaid, 1999).

Feeding of minerals has extreme importance in maintenance and production of healthy sheep and lambs. Grazing sheep in west Asia usually receive mineral supplementation and must depend almost exclusively upon pasture, crop residues and grain since they raised as an adjunct to crop production area (Abdelrahman, 2003).

A study by Abdelrhman (1998) for grazing dairy Cattls, found a seasonal changes in concentration of P, Cu and K, Ca, Mg, Co and Zn in their blood serum. Concentration of P, Ca and Na in serum were the lowest during the late dry season.

In another study at northern part of Jordan, a seasonal change profiles in minerals (Ca, P, Mg, Cu, Zn, Mn, and Co) in serum of grazing sheep at northern part of Jordan was detected (Abdelrahman, 2003). Moreover, in a study for mineral status of 230 cattle and 175 sheep in northern part of Jordan, was determined by liver analysis for P, Ca, Mg, Zn, Fe, and Cu. Clinical signs of suspected Cu, Zn, Ca, P, and Mg deficiencies were observed (Albel *et al.,* 1979).

Moreover, a study of mineral and vitamin status of sheep in Syria, Jordan and Turkey was conducted. Blood samples were collected from 18 sites during the winter period of 1994. The results indicated a deficiency in Vitamin E, Cu and Zn levels. Vitamin B12 concentrations were above the deficient range at all sites. Moreover, the results indicated that sheep at risk of mineral and vitamin deficiencies in Syria, Jordan, and turkey. They recommended further studies to be conducted in the region as well as extended to North Africa and Eastern Asia (White *et al*., 1995).

Selenium is an essential nutrient with many potential health benefits for humans, including protection against some cancers, enhancement of neuropsychological function, and maintenance of a healthy immune system.

Dietary Se is present in a variety of chemical forms and many of its biological actions depend on the chemical form of Se that is consumed (Finley, 2000).

Zinc has been shown to be essential for microorganisms, plants, and animals. Deprivation of zinc arrests growth and development and produces system dysfunction in these organisms. The biological functions of zinc can be divided into three categories: catalytic, structural, and regulatory (Kincaid, 1998). Zinc is the most abundant intracellular trace element. It is ubiquitous in the body and is required for the function of more than 200 enzymes, as well as DNA-binding proteins, and thymic hormones. It is sometimes called an antioxidant because of its requirement for antioxidant enzymes (Halliwell and Gutteridge, 1991).

Copper plays an important role in tissue oxidation and its deficiency occurs primarily in young ruminants. The type of diet, the presence of other substances (molybdenum, sulfur and iron) in the diet and genetic constitution of the animals, influences the absorption of copper (Klotz *et al,* 2003).

Manganese was shown to be essential in 1931 when this element was found to be necessary for growth and reproduction in rats and mice, since that time manganese has been shown to be essential for many species of animals. Recently, the possible occurrence of manganese deficiency in man has been reported (Leach, 1983).

In general, winter to early spring is a good time for routine monitoring or investigations to determine the probability of occurrence of a significant deficiency of the four trace element Copper, Selenium, Zinc and Cobalt (Seyler *et al*., 2003).

Diet analysis provide useful supporting data if representive samples of all feeds can be obtained. Actual chemical analyses need to be performed and should include those elements with important interaction (Kincaid, 1999).

Deficiencies and imbalances of trace elements can affect productivity of ruminants. Whereas improvement in feed intake can occur quickly in response to zinc or cobalt supplementation, productivity responses to copper or selenium may be slower to become evident by improved health of newborns, greater resistance to mastitis, or increased weaning weights of calves (Kincaid, 1999). An animal's trace element needs increase during pregnancy, lactation and growth (Galloway *et al.*, 2000).

Defficiencies of the mineral can result in poor growth and reproduction wasting disease, disorder, non-infections abortion, anemia, bone abnormalities, tetany, and many other disorders (Underwood, 1981). The trace mineral concentration in plants is reduced by the dilution effect of

rapid growth during spring. Higher levels of sulphur in green feed may reduce the availability of copper and selenium (McDowell, 1985).

The trace elements have strong correlation with some enzyme such as antioxidant enzymes Glutathione peroxidase (Gpx), Glutathione Reductase (GR), Glutathione-S transeferase (GST), and Catalase (CAT). These enzymes play major large role in body health in both animals and human.

Most of authors have established a strong positive correlation between quantity of selenium and glutathione peroxidase (Gpx) activity, while the glutathione peroxidase activity stated has been very uneven (Harapin *et al*., 2000). Concentrations of Se and activities of Gpx are highly correlated ($r = .92$; $P < .001$) in blood of sheep and cows ($r = .59$, $P < .001$) (Kincaid, 1999). Trace element play role in activity of antioxidant enzymes, such that their levels in liver and blood is altered with changes in the activity of these enzyme. In addition, trace elements may function as cofactors for enzymes, or stabilizers of secondary molecular structure. Their function has evolved from recognition of their essential function in cell metabolism. There has been special interest in the effects of dietary trace element deficiencies on physiological function and reproduction. Severe dietary deficiencies of trace elements including copper, selenium and zinc are commonly seen in ruminant's studies (Naziroglu *et al*., 1998).

Deficiencies of mineral depend on the type of the pastures and climate. So, more studies are needed to know where and when trace elements deficiencies occur and if a supplementation program trace elements is needed in specific season to improve livestock productivity.

The broad goal of this study was to assess the trace element status and enzyme activities of grazing Awassi sheep. Moreover, trace elements concentrations in soil and feeds will be determined at different locations in Al-Karak region throughout the year.

1.2. The specific objectives of this study were to:

1). **Determine the seasonal variations in trace element levels Copper (Cu), Zinc (Zn), Manganese (Mn), Cobalt (Co), and enzymes activities by analyzing blood antioxidant enzyme that related to variation in trace element level by season, such as Glutothione peroxidase (GSH_{px}) Glutathione reductase (GR), Glutahione S-Transferase (GST) and Catalase of in whole blood of grazing Awassi sheep.**

2). **Trace minerals concentrations in feeds (forages, cereals, crops), and soil at different locations in Al-karak region.**

3). Determine and correlate the annual changes in trace elements and other profiles of Awassi sheep blood with the seasonal availability of mineral nutrients.

4). Effect of seasons and locations on the concentration of trace elements and enzymes activity.

Chapter Two

Review of Literature

2.1 Trace element

Minerals play substantial role in sheep nutrition; certain minerals are essential in the diets of animals and influence livestock production (Mc Dowell, 1985; Kincaid, 1988). Those minerals required in significant amount in micrograms and milligrams per kilogram of dry matter ingested, they include selenium, manganese, zinc, copper, cobalt, molybdenum, iron and iodine (Galloway *et al*., 2000). They constitute a relatively small amount of the total body tissues; but they are essential to many vital processes. Increased interest in biological aspects of metals has come with the establishing of sophisticated instruments, industrial revolution and exposure of men to metals in an occupational setting and recognitions of toxic states (Mumtaz *et al.,* 1998).

Mineral are derived principally from forage, soil, and water. Although highly variable, all mineral elements essential as dietary nutrients occur to some extent in water (McDowell, 1985).

Several trace elements such as selenium, zinc, copper, manganese, etc have immuno modulatory functions and thus influence the susceptibility to the course and the outcome of a variety of viral infections (Kincaid, 1999). Those have particular functions in specific biological processes and for the most part are unalterable. A longer absence cannot be compensated for and leads to decreased metabolic reactions with typical signs of deficiency after a period of several weeks. Zinc, copper, selenium, chromium, and molybdenum are comparable trace elements with changing valences and, consequently, a wide variety of different tasks in biological organisms and currently changing bioavailability (Galloway *et al*., 2000).

The trace mineral status of animals is best described by concentrations in liver. Correlation coefficients between concentrations of trace minerals in blood and liver are highest in deficient animals because endogenous reserves are depleted. Concentrations of Zn, Cu, and Se in plasma also are affected by infection, stress, pregnancy, and erythrocyte hemolysis (Kincaid, 1999). The concentration of mineral elements in plants from diverse world regions are dependent upon the interaction of a number of factors including soil, plant species, stage of maturity, yield, pasture, and climate (McDowell, 1985).

The high level of soil fertility, the lower the consumption of minerals (Underwood, 1981). Most naturally occurring mineral deficiencies in livestock are associated with specific regions and directly related to both soil and mineral concentration and soil characteristics (McDowell, 1985). The soil is ingested by grazing animals indicated that not only the usual sequence of soil- plant- animal relationship occurred, but also a direct soil- animal effect should be considered (McDowell, 1985).

Trace element plays an important role in human health and disease. Through their participation in tissue, cellular and sub cellular function. These include immuno regulation by normal and cellular mechanism, nerve conduction, muscle contraction, membrane potential regulation, and mitochondrial activity (Mumtaz *et al*., 1999). Moreover, trace element and toxic metals concentration in livestock is important for assessing the effect of pollutant on domestic animals and contaminant intake by humans (Alonso *et al*., 2000). Some metals ions e.g. cobalt, can also decomposes lipid peroxides. However, neither zinc nor manganese is effective, and both zinc and manganese salts have been reported to inhibit lipid peroxidation in some systems (Halliwell and Gutteridge, 1991).

The oxidative hemolysis could be associated with reduced level of antioxidant enzymes (e.g. superoxide dismutase (SOD) and glutathione peroxidase (GPX) and some trace elements, which serve either cofactors for these enzymes or integral parts of zinc, copper, and selenium (Oshiro *et al.,* 2001). The concentration of metals ions may limit the rate of decomposition of lipid peroxides in vivo (Halliwell and Gutteridge, 1991).

In metalloenzymes, the metal is firmly attached to the protein moiety, with a fixed number of metal atoms per mol of protein. The metal cannot be removed without loss of enzyme activity and usually cannot be replaced by any other metal (Underwood and Suttle, 2001).

2.1.1. Selenium

2.1.1.1. Definition

Selenium (Se) was discovered by Berzelius in 1818 and for the next 140 years the toxicity of Se was the main concern for biologists. In 1950s Se was considered to be a highly toxic element with possible carcinogenic properties (Wachowicz *et al*., 2001). It is an essential trace element of sufficient clinical relevance to warrant the monitoring of its status in selected patients. It's a metalloid element, located in-group VI of the periodic table. Its biochemistry, toxicology and nutritional importance have been reviewed regularly and thoroughly over the last decade (Sheehan *et al*., 2001).

2.1.1.2. Biological function of selenium:

Selenium and other trace element play important role in animal husbandry. These element help to minimize infectious disease reduce concomitant stresses and bring about optimal reproductive performance (Pamukcu *et al*., 2002). Selenium (Se) has always been regarded as an important element in animal nutrition because of its toxicity. In 1957, the role of Selenium nutrition assumed a new aspect when it was demonstrated that feeding with extremly small amounts (0.5mg/kg) of Se as sodium selenite prevented liver necrosis in rats (McDonald *et al*., 1987)

Selenium and free radical:

Free radical usually has a single electron (unstable) in the outer orbital instead of two electrons (stable form). Free radical production normally in the biological system and necessary part of normal cell function (Fantone and Ward, 1982).

There are large number of enzymes and cofactor involved in the natural defense mechanism against free radical. Some of metal antioxidant factors are Se in glutathione peroxidase, Cu- Zn- Mn in superoxide dismutase, Fe in Catalase, Fe-Mn in aldehyde dehydrogenase. Glutathione peroxidase, SOD, and catalase are the main intercellular enzymes that provide protection against the free radicals of oxygen. Glutathione peroxidase, an Se dependent enzyme, catalyses the conversion of peroxidases to hydroxy compounds (Karlmark, 1993).

Other function of selenium:

It is possible that selenium plays other biochemical roles, including an involvement in haem metabolism and observed protection against the action of certain carcinogens. It has been suggested that low blood selenium might predispose to cardiovascular disease or to complications of pregnancy (Halliwell and Gutteridge, 1991). It's necessary for the development of acquired immune system; Se suggested playing a role in the preventing of certain forms of cancer, playing an important role in thyroid hormone activation (Karlmark, 1993).

The importance of cancer as a leading cause of death in humans has stimulated a great deal of research attempting to link selenium status to incidence or severity of cancer, in both animals and humans (Wachowicz *et al*., 2001). On the other hand, mechanisms of anticarcinogenic effects of Se compounds appear to include not only the

expression of selenoproteins but also pharmacological actions of Se metabolites (Halliwell and Gutteridge, 1991).

2.1.1.3. Deficiency:

Number of diseases or production of inefficiency syndromes are associated with selenium and/ or vitamin E deficiency. In most of these syndromes selenium and vitamin E appear to be complementary but in terms of recognized deficiencies and response to treatment selenium is the most important (Hoekstra, 1974).

Selenium deficiency in animal's results in growth retardation, reproductive failure and degenerative organ changes (Naziroglu *et al*., 1998). Several diseases have been related to oxidative stress. Recently, antioxidant functions have also been linked to anti-inflammatory properties. Cell against reactive oxygen species include antioxidant enzyme (Mates *et al*., 1999). Selenium deficiency could also have negative impact on immune system function and other organ functions vital for recovery from infectious disease (Klotz *et al.*, 2003).

In the last years, there has been a renewal of interest in the protective role of selenium in vascular disorder; a relationship has been established between a decrease in plasma selenium and an increase in the risk of coronary disease (Vitoux *et al*., 1996). Another condition that may be selenium-responsive is the tragic problem with newborn infants, which has been referred to as crib death or sudden infant death syndrome (SIDS) (Naziroglu *et al*., 1998). The severity of live injury is a factor conditioning the impairment in the selenium body status observed in individuals with hepatopathies (Alracon *et al.*, 2001).

In humans, selenium deficiency is thought to be involved in Keshan disease, a cardiac myopathy and, possibly, Kashin-Beck disease a condition of the joints and muscles (Naziroglu *et al*., 1998). Occurring in the Keshan region of China where selenium intake is extremely low. The main clinical features are acute or chronic episodes of heart disorder characterized by cardiogenic shock and/or congestive heart failure (Wachowicz *et al*., 2001).

2.1.1.4. Interrelationship in soil, plant, animals and human with season:

2.1.1.4.1. Soil associations

The selenium content of most soils ranges from 0.1 to 2 parts per million. In general, soil selenium content by itself is not a good measure for the potential for occurrence of selenium deficiency in livestock grasses or consuming forages produced from it. Soil pH has a marked effect on the form of selenium present and its availability to the plant. In alkaline, well-

aerated soils with low rainfall selenium forms selenates and organic selenium compounds with good availability to plants (pastrana *et al*., 1991).

Plant growing in acidic soil does not efficiently take up selenium from the soil, so areas that are acidic with low levels of selenium in the soil will produce forage with low concentration (Karlmark, 1993).

2.1.1.4.2. Plant associations

The selenium content of plant varies widely and is influenced by the species, Se form in soil, and many other factors related to the soil (NRC, 1983). Seasonal conditions influence the amount of selenium in plants and when climate favors a lush forage growth deficiency can occur. Consequently, pasture levels tend to be lowest in the spring when the pastures are growing the fastest. Heavy grazing of pastures may also deplete selenium in the plant (Pastrana *et al*., 1991).

Plants are known to convert inorganic Selenium in the soil to organic Selenium compounds via the sulphur assimilatory pathway. Selenized yeast contains a cocktail of Selenium in a variety of chemical forms, with SeMet as the major constituent (Wachowicz *et al*., 2001). In soils with very low selenium levels the plants produced are likely to be selenium deficient and the more acid soil the more likely the deficiency (pastrana *et al*., 1991). The level of Se in plants and in turn in animals depends on the amount of biologically available Se in the soil where Se content varies greatly (Wachowicz *et al*., 2001). Seasonal and possible pasture species variations should be taken into account when pasture analysis is attempted. Complete mixed rations analyses are simpler. The minimum requirement for selenium for ruminants is usually placed at 0.1 to 0.2 parts per million of the total ration on a dry matter basis. These should be considered minimal values, as deficiency states can exist at this level of intake (Sprinkle *et al*., 2000).

The trace mineral concentration in plants is reduced by the dilution effect of rapid growth during spring. Higher levels of sulphur in green feed may reduce the availability of copper and selenium (Yokus *et al*., 2004).

In particular sulfur-containing fertilizers depress selenium uptake by plants. Plant selenium concentrations of less than 0.1 parts per million may be associated with selenium deficiency and concentrations of less than 0.05 have a higher probability. If pasture samples are to be analyzed for selenium content the variation with pasture species and with season must be taken into account (Sprinkle *et al*., 2000).

2.1.1.4.3. Selenium in animals:

Most selenium in animal tissues is present as selenomethionine or selenocysteine. Selenomethionine, which cannot be synthesized by humans and is initially synthesized in plants, is incorporated randomly in place of methionine in a variety of proteins obtained from plant and animal sources. Selenium is present in varying amounts in these

proteins, which are called selenium-containing proteins (Halliwell and Gutteridge, 1991).

In humans, acute selenium toxicity is characterized by gastrointestinal disturbance, hair loss, and numbness in the arms, fatigue and garlic-smelling breath. In China where endemic selenosis occurs, symptoms such as brittle and pigment less hair, skin lesions, pathological changes to the nails and neurological disturbances are observed (Halliwell and Gutteridge, 1991).

2.1.1.5. Methods for selenium analysis:

Several methods for determination of selenium in biological fluid have been developed, since the discovery of selenium as an essential element by (Schwarz and Flotz, 1957).

Atomic absorption spectrophotometry (AAS):

Determination of selenium by hydride generator AAS were developed to improve the sensitivity and accuracy. This method involves the reduction of Se^{+4} by sodium tetraborohydride ($NaBH_4$) in hydrochloric acid solution (Campell, 1992).

Neutron activation analysis (NAA):

Selenium present in biological sample in low concentration but the radioactivity of Se is affected by activities of other radionucleides, such as ^{24}Na, ^{82}Br, ^{38}Cl… etc. (Lavi and Alfassi, 1989). Through the long- lived radionucleide ^{75}Se (120 days) or by using the short- lived ^{77m}Se (Wortties and Nieumendijk, 1987).

Indirect assessment:

The discovery of glutathione peroxidase (GSH-px) as a Se-dependent enzyme was made by Rotruk and other in 1973. Selenium levels can be measured in plasma or serum, whole blood, red cells, hair and nails. Measuring tissue GPX activity can also assess selenium status. When growing animals are fed a selenium deficient diet, a rapid drop in GPX activity occurs, suggesting that selenium stores are being depleted (Halliwell and Gutteridge, 1991). Blood is the most convenient animal material for examination. Analysis can be made for selenium or for the selenium containing enzyme glutathione peroxidase. Liver and kidney can be used for analysis of selenium status but unlike copper deficiency probably offer no advantage to blood analysis, because they are significantly correlated to nutritional status of some trace elements (Kincaid, 1999). For serum or plasma, it is important to avoid

haemolysis as this enriches the selenium content and increases iron concentration markedly, making specimens unsuitable for analysis. Samples should be stored refrigerated prior to analysis; repeated freezing and thawing of samples can denature proteins, which will also affect results (Sheehan *et al,* 2001).

Measures for estimating the Se status of livestock include concentrations of Se in liver, serum, and whole blood; glutathione peroxidase (GPx) activities in erythrocytes and liver; and mRNA levels for GPx or hydroperoxide glutathione peroxidase (Kincaid, 1999).

Concentrations vary substantially in healthy subjects, mainly according to geographic location, diet and age. The mean serum Se concentrations for healthy adult subjects in different parts of the world vary from 40 to 200 µg/l. (Wachowicz *et al*., 2001). In hepatic patients serum total cholesterol level showed a significant positive correlation with serum selenium concentration demonstrating the important role of selenium as an antioxidant agent (Alarcon *et al*., 2001).

Selenium deficiency in animals produces a variety of diseases that are strikingly similar to those induced by vitamin E deficiency (Halliwell and Gutteridge, 1991).

2.1.2. Copper:

2.1.2.1. Important of copper:

Copper essentially was obtained in 1924, when experiment with rats showed that copper was necessary for haemoglobin formation (Mc Donald, 1985). Copper is an essential element for ruminants and its deficiencies occur in grazing animals in many parts of the world. Cu joins in formation of many enzyme systems and thus its deficiencies may be reflected in those metabolic and clinical symptoms related to these enzymes. Cu deficiency causes infertility in ruminants (Naziroglu *et al*., 1998). In plasma it is present mainly in two forms, loosely bound to albumin and tightly bound to ceruloplasmin. Ceruloplasmin (Cp) is a blue copper protein resulting form a combination of Cu with alpha macro-globulin. Every Cp molecule contains eight atoms of Cu. Ninety percent of plasma Cu is in the Cp form (Underwood, 1981).

2.1.2.2. Copper deficiency:

Copper is very important for CNS and its deficiency could cause CNS abnormalities. The others clinical manifestations of Cu depletion are: anorexia, failure to grow, diarrhea, pallor, depigmentation of hair and skin, dilated superficial veins, defective elastin formation (aneurysm),

hypothermia, osteoporosis, periosteal reactions, cupping and flaring of long bones, flaring of anterior ribs, sub metaphyseal fractures (McDowell, 1985).

Copper deficiency may occur as a simple deficiency where the concentrations of copper in the diet are markedly deficient. Copper deficiency can also occur as a conditioned deficiency and in this situation copper concentrations in the diet may be marginal to normal but other constituents of the diet interfere with absorption and utilization of the ingested copper. Prominent amongst these are molybdenum, sulfur and iron (Underwood, 1981). May be primary, when the intake in the diet is inadequate (forage is grown on deficient soils or on soils in which the copper is unavailable) or secondary when the dietary intake is sufficient but the utilisation of the copper by tissues is impeded (Kojouri, 2002).

Young lambs are very sensitive to Cu deficiency. Osteoblastic activity is low and abnormal bones result in young lambs born to Cu deficient ewes (Suttle and Jones, 1989). Plant associations with copper deficiency are complex because of the interaction between copper and other conditioning substances on the availability and absorption of copper by the ruminant (Albel *et al.,* 1979). Copper required for growth and production, animal health and immunity action reproduction. High intakes of Mo, S and Fe, which often occurs in pasture-based diets, especially in winter and spring, reduces copper uptake by the animal (Galloway *et al*., 2000). Loss of pigmentation occurs with intakes of Cu that are sufficient for pregnancy maintenance and hemoglobin formation. Pregnancy is not maintained by intakes of Cu that prevent anemia (Kincaid, 1999). Pasture analysis and knowledge of factors that may affect subsequent copper intake and availability are of value in predicting the possible causes of this status. The liver is the main store of copper in the body (McDowell, 1985). Pasture sampling cannot be used to diagnose the occurrence of copper deficiency in cattle. Pasture analysis can be used to determine the nature of the copper deficiency, for example whether it is a primary deficiency or secondary due to high molybdenum and/or sulfate intakes (Underwood, 1981).

Animals of any age can be tested but animals which have grazed dry pasture (over summer) are less likely to be deficicient than young animals born in late autumn, winter, or spring (Sprinkle *et al*., 2000).

2.1.2.3. Copper correlation with soil, plant, animals and human:

In general the copper content in grasses ranges from 4-9 parts per million dry matters. Legumes generally have higher concentrations of copper than grasses under similar growing conditions. Pastures with less than 5 parts per million of copper may be associated with copper

deficiency especially if there are significant levels of molybdenum and sulfur (McDowell, 1985).

High dietary intakes of Molybdenum (Mo), Sulfur (S), Zinc (Zn), Cobalt (Co) and Cadmium (Cd) and soil all decrease the availability of dietary copper. Molybdenum application to pastures can exacerbate a copper deficiency (Naziroglu *et al.*, 1997). Pastures with less than 2 ppm coppers may be associated with primary copper deficiency. The availability of copper to the animal varies with the type of plant and the stage of maturity. Availability is highest in the mature pastures and in hay and lowest in rapidly growing young pastures but silages are intermediate (Underwood and Suttle, 2001). The relative availability of copper from herbage is lower in winter than in summer (Naziroglu *et al.*, 1997). Which animal grazed dry pasture (over summer) are less than young animals born in late autumn, winter or spring (Sprinkle *et al.*, 2000).

2.1.3. Zinc:

2.1.3.1. Zinc Important and function:

Zinc is an essential trace element that primary can be found intracellular. Only 0.1 % of the body zinc can be found in the plasma. There are no specific storage sites known for zinc so a regular supply in the diet is required. Zinc in the muscle and the bones to some extend can be reused to avoid deficiency (Galloway *et al*., 2000).

Zinc has an important responsibility in numerous biological human functions, as a catalyst for many enzymes - related metabolic processes and as an essential part of some specific proteins. It participates in the synthesis of nucleic acids and proteins and helps to protect the integrity of biomembranes (Galloway *et al*., 2000).

Zinc has an important responsibility in numerous biological human functions, as a catalyst for many enzyme related metabolic processes and as an essential part of some specific proteins (McDowell, 1985).

2.1.3.2. Zinc deficiency:

It has a decisive influence on the immune reaction, predominantly in lymphocyte proliferation and differentiation. As a result, zinc deficiency is often associated with a decline in enzymatic and immunological reaction, which frequently improves dramatically after zinc replenishment (Halliwell and Gutteridge, 1991). It was found that Zn aspartate is an efficient inhibitor of the formation of the most reactive hydroxyl radicals. These antioxidant properties of Zn aspartate make it important in medicine for the prevention

and treatment of free radical pathologies (Fisberg *et al.*, 1999). Many diseases and clinical syndromes have proved to be associated with diverse presenting features of zinc deficiency. Diseases recognized to be complicated by zinc deficiency include malnutrition, a variety of intestinal diseases such as Crohn's disease, sprue, alcoholic liver disease, and sickle cell anemia (McDowell, 1985). In human metabolism, zinc ions are key structural components of a large number of proteins with highly specific functions. This in particular underlies the significance of zinc in comparison to other essential trace elements (Galloway *et al.*, 2000). Associated with individuals having a diet low in meats resulting in generalized metabolic impairment because of zinc's widespread role as a cofactor. Severe deficiency can impair immune function, digestive function. In children it can lead to mental retardation and arrested sexual maturation. Groups mostly at risk for deficiency include pregnant women, children, the elderly and HIV patients (Galloway *et al.,* 2000).

2.1.3.4. Zinc and enzyme activity:

The number of zinc enzymes for which structural data are available is increasing rapidly. More than 300 enzymes require zinc in various intensities and with different specificities, whether as an indispensable structural part of the enzyme molecule, or as catalytic factors or coactive associates (McDowell, 1985). Such as Superoxid dismetase (Cu-Zn-SOD), Carbonic anhydrase, pancreatic oxypeptide and Glutamic dehydrogenase and number of Pyridine nucleatide dehydrogenases (McDowell, 1985). In addition, Zn is a cofactor of many metalloenzymes that are involved in numerous biochemical processes in the body such as skin integrity, immunity, impaired taste, poor wound healing, delayed sexual maturation, growth retardation, weight loss, abnormal dark adaptation, conversion of oxygen, protein synthesis, protein degradation, metabolism of carbohydrates, metabolism of vitamin A and defence against free radicals (Kargin *et al.*, 2004).

2.1.4. Manganese

Manganese was shown to be essential in 1931 when this element was found to be necessary for growth and reproduction in rats and mice. Since that time manganese has been shown to be essential for many species of animals. Recently , the possible occurance of manganese deficiency in human and animal was obseved (Leach, 1974). Manganese present in the animal body is extremly low conecentration. Most tissues contain traces of this element, the highest concentration occuring in the bones, liver, kideney,

pancreas and pituitary gland. Manganese is important in the animal body as an enzyme activator and resembels magnesium in activating anumber of phosphate transferase and decarboxylases, notably those concerned with the tricarboxylic acid cycle. symptoms of Manganese deficiency were first noted in rats fed on purified diets (McDowell, 1985). Becuase of the very low concenration of Mn in blood, determine of Mn is very difficult (Hills, 1974).

2.1.4.1. Important of manganese:

Most of workers agree that manganese (Mn) is an essential element for all higher animals, abetting survival, growth, and reproduction. The biological availability of Mn is determined by many factors: PH, valence of Mn, type of chelating agen present, pattern and amounts of other elements. Each factor may alter the availability of Mn^{2+} (Doisy, 1974).

The animal grew slowly and bone structure and reproduction were affected. The reproductive failures were quite marked and included defective ovulation in females and testicular degeneration and sterility in males (McDonald *et al*., 1985). Manganese induced a significant increase in the cholesterol of blood and brain tissues. The manganese induced learning disability corrected completely. This correction led to conclude that manganese by increasing hippocampal cholesterol levels impairs learning ability (Oner and Senturk, 1992).

2.1.4.2. Enzymatic role of manganese:

The relationship between manganese and enzymes can be classified into two categaories: (a) metalloenzyme, and (b) metal- enzyme complex (Leach, 1974). Manganese unlike other element such as Cu, Zn, the number of manganese metalloenzymes is very limited. But the enzymes that can be activated by manganese are numerous (Leach, 1974).

2.1.5. Cobalt (Co):

2.1.5.1. Important of Cobalt:

In 1930, discovery of the need for Cobalt (Co), grazing ruminants could not be produced in many regions due to deficient concentrations of this element in forages; Co was effective in preventing two-delitiating disease of sheep known as " coast disease" (McDowell, 1985). It is widely distributed in the environment, accounting for 0.001% of the earth's crust. It forms bivalent and trivalent compounds, those of biological interest being bivalent (Hosking *et al.*, 1986). Inorganic sources of cobalt must be partially soluble in the rumen to be of nutritional value to ruminant when used as food supplements (Underwood and suttle, 2001).

Cobalt is commonly stated to be of low toxicity to all species (Underwood, 1981). Grazing ruminants could be produced in many regions due to deficient concentration in forages (McDowell, 1985). As essential trace element being an integral part of vitamin B_{12}, which is essential for folate and fatty acid metabolism (Underwood and sutlle, 2001). Although cobalt is an essential trace element, cobalt deficiency has not been reported in humans. A wasting disease in cattle, of which a key feature is anemia, has been demonstrated to be due to cobalt deficiency in pastures (Garton *et al.*, 1981). The perinatal mortality and morbidity can be high amongst lambs born for ewes received an inadequate intake of cobalt for some time before, and during pregnancy (Garton *et al.*, 1981).

The seasonal variations in cobalt nutrition are significant. The concentration of cobalt in pastures and of B_{12} in plasma is lowest in spring. There are also large fluctuations in the availability of cobalt between years. Seasons with lush pasture growth favour the development of cobalt deficiency perhaps because the sheep ingest less soil. Soil provides a more concentrated source of cobalt to the ruminant (Hosking *et al.*, 1986).

2.1.5.2. Cobalt deficiency:

Cobalt deficiency can be prevented by taking animals every year for a few months to "healthy" region, preferably during the rainy season (McDowell, 1985). In cobalt deficient lambs the total concentrations of rumen bacteria and the principal types of those organisms were reduced below normal (Underwood, 1981). Deficiency signs of cobalt are not specific, and it is often difficult to distinguish between an animal having a deficiency due to low intake of energy and protein (McDowell, 1985). Under grazing conditions, lambs are the most sensitive to Co deficiency, followed by mature sheep (Underwood, 1981).

Acute clinical signs of cobalt deficiency include lake of appetite, rough hair coat, thickening of the skin, anemia (McDowell, 1985). Eventually failure of growth or weight loss (Underwood and sutlle, 2001).

2.2. Antioxidant enzyme:

2.2.1. Glutathione peroxidase (GP_x):

Glutathione peroxidases (GPx) are a family of enzymes that can be divided in to two groups, selenium–independent and selenium-dependent enzymes. The latter group can also decompose H_2O_2 and various hydro- and lipid peroxides (Halliwell and Gutteridge, 1991). Glutathione peroxidase is a

kind of Se-dependent enzyme. It is an antioxidant that involves in defense against peroxidation and prevents the breakdown of hydroperoxides from formation of new radical. The selenocysteine residue in this protein is the basic catalytic process in the function of GPx. The kinetics of GPx studies under steady conditions shows the Ping Pong mechanism (Klotz *et al*., 2000). It's one of the most important antioxidant enzymes in humans, demonstrated that normal pregnancy is associated with increased GP_x activity and insulin resistance (Chen *et al*., 2003). GP_x react with H_2O_2 and fatty acid hydro peroxides, simultaneously oxidizing GSH to GSSG, in the presence of excess glutathione peroxidase, the rate of NADPH consumption can be related to the peroxide content of the system (Halliwell and Gutteridge, 1991). The hydro peroxide is subsequently reduced by the seleno enzyme glutathione peroxides (GP_x). Cytosolic GP_x can also act as aperoxynitrate reductase (Klotz *et al*., 2000). Most free glutathione in vivo is present as GSH rather than GSSG, but up to one-third of the total cellular glutathione may be present as mixed disulphide with other compounds that contains- SH groups, such as cystiene, coenzyme A, and the SH of the cystiene residues of several proteins (Halliwell and Gutteridge, 1991).

Selenite (selenate after reduction) enters cells, and reacts with thiols; glutathione GSH is considered to be the main component of the Se metabolism pathway taking part in the first of a series of reduction reactions, which convert selenite to hydrogen selenide (H_2Se). GS-Se-SG (seleno diglutathione) is the major product formed (Wachowicz *et. al*., 2001). GSH is not only a substrate for transferase enzymes, but can also often combine directly with the ions or radicals that attack DNA (Halliwell and Gutteridge, 1991). Selenium as selenomethionine was more effective than selenite in maintaining Se concentration and GP_x activities blood (Awadeh *et al*., 1998). Blood is the most convenient animal material for examination. Analysis can be made for selenium or for the selenium containing enzyme glutathione peroxidase (Gay, 2003).

The ratio of GSH/GSSG in normal cells is kept high, so there must be a mechanism for reducing GSSG. This is achieved by glutathione reductase enzymes, which catalyses the reaction:

$$GSSG + NADPH + H^+ \longrightarrow 2GSH + NADP^+$$

In this reaction reduced glutathione (GSH) is used as a cosubstrate to metabolize H_2O_2, resulting to H2O and oxidized glutathione (GSSG). Oxidized glutathione (GSSG) can be reduced back to GSH by the enzyme GSH reductase (GR), a reaction requiring NADPH regenerated by glucose 6-phosphate dehydrogenase (Figure 1) (Halliwell & Gutteridge, 1991). Glutathione (GSH) acts as an antioxidant by destroying H_2O_2 via glutathione

peroxidase that requires selenium and by replenishing reduced vitamin C. Glutathione (GSH) is restored via glutathione reductase using NADPH (Wachowicz *et al*., 2001). Moreover, glutathione peroxidase (Gpx) acts to destroy peroxides before they attack cell membranes while vitamin E acts within the membrane to attract unsaturated fatty acid molecules and form loose chemical complexes until they are metabolized during cell respiration, thus preventing the formation of fatty acid hydro peroxides (Halliwell & Gutteridge, 1991).

GSH is considered more important than catalase in physiologic conditions for several reasons. The enzymes of the GSH redox cycle are distributed throughout the cytosol; the Km value is lower for GPx than for catalase suggesting the preferential pathway for the degradation of low concentrations of H_2O_2 present in intact cells. Moreover, the GSH redox cycle has the capacity to metabolize hydro peroxides other than H_2O_2 (Oshiro *et al.*,2001). GSH is important in maintaining membrane integrity particularly in the face of an "oxidant stress" imposed by the various drugs such as primaquine, sulfanilamide, etc. GSH is believed to protect the cell by its role in peroxide decomposition and possibly by maintaining protein-SH group's SH-SS inter change (Hoekstra, 1974).

2.2.1.1. Biological function

$$ROOH + 2GSH \xrightarrow{GPx} ROH + H_2O + GSSG$$

Unspecific for hydroperoxides. Can be about anything from H_2O_2 to peroxidized membranes and DNA. Specific for GSH. Similar compounds have much less reactivity. It yields a single oxidation product, in contrast to heme peroxidases (Hoekstra, 1974).

Selenite (selenate after reduction) enters cells, and reacts with thiols; glutathione GSH is considered to be the main component of the Se metabolism pathway taking part in the first of a series of reduction reactions, which convert selenite to hydrogen selenide (H_2Se). GS-Se-SG (Selenodiglutathione) is the major product formed. A consequence of the reaction of selenite with glutathione is the production of H_2O_2 and $O2^{-} \cdot$ (Wachowicz *et al*., 2001).

Further study has revealed that several animal tissues contain a non-selenium glutathione peroxidase activity that act on artificial organic hydro peroxides but not on hydrogen peroxide. This activity appears to be due to some of glutathione-S-transferase enzymes involved in the conjugation of GSH with foreign compounds (Halliwell and Gutteridge, 1991). However, environmental factors have also definite effect on enzyme action. Nutrition

is one of the most essential factors as fat content and fatty acid composition of feed, or trace element intake as well as vitamin status of the animal play crucial role in normal enzyme activity. Seasonal changes have also some effect on Gpx activity as circannual changes have been reported in the literature (Mezes *et al*., 2003).

In study by Hunaiti *et al,* (2000), lowered blood glutathione content was observed in occupationally lead- exposed workers. The reduction of blood glutathione associated with incubation of whole blood lead salts could result from direct interaction of lead with glutathione synthesis and regeneration.

2.2.2. Glutathione Reductase (GR):

Glutathione reductase contains two protein subunits each with the flavin FAD at its active site. Apparently the NADPH reduced the FAD, which then passes its electrons on to a disulphide bridge (-S-S-) between cysteine residues in the protein. The two -SH groups formed then interact with GSSG and reduced it to 2 GSH, reforming the protein disulphide (Halliwell and Gutteridge, 1991). Glutathione reductase is a member of an important class of flavoprotein enzymes, the disulfide oxidoreductases, containing two active-site electron acceptors, which are FAD and a redox-active disulde (Loprasert *et al.,* 2005).

2.2.2.1 Biological function

$$GSSG + NADPH + H^{+} \xrightarrow{GR} NADP+ + 2GSH$$

Same assay as GPx, measure NADPH, other substrates besides GSSG: only mixed disulfides between GSH & γ-glutamylcysteine or CoA (Halliwell and Gutteridge, 1991). Biologically, removes GSSG, which is toxic. Keeps GSH in reduced form so it can be used (Figure 1). There are families with low levels of GR in red cells. Under normal circumstances, but under oxidative stress, red cells hemolyze. Location in eukayrotic cell: cytoplasm, mitochondria (Wachowicz *et al*., 2001). Oxidized glutathione (GSSG) can be reduced back to GSH by the enzyme GSH reductase (GR), a reaction requiring NADPH regenerated by glucose 6-phosphate dehydrogenase (Halliwell and Gutteridge, 1991).

2.2.3. Catalase (CAT)

Catalase is a protein of molecular weight approximately 240.000, which contains 4 ferriprotophyrine (hematin) groups (Ben-yosebh and Shapira. 1973). Where found in animals in all major body organs, being especially concentrated in liver erythrocytes (Halliwell and Gutteridge, 1991). Some

organisms containing Mn- Catalase enzyme that is important in protecting this organism against H_2O_2 (Halliwell and Gutteridge, 1991). Each sub unit also usually contains one molecule of NADPH bound to it, which helps to stabalize the enzyme (Halliwell and Gutteridge, 1991). Catalase found in liver, kidney, blood and mucous membranes which often localized in peroxisomes of the cell. As noted above, hydrogen peroxide is produced by the catabolism of superoxide-by-superoxide dismutase (Ben-Yoseph and Shapira, 1973).

2.2.3.1. Enzymatic functions

$$2\,H_2O_2 \xrightarrow{\textbf{CAT}} 2\,H_2O + O_2$$

Biological Functions of catalase that removes H_2O_2, adds O_2 and protects against lipid peroxidation, may participate in alcohol metabolism (Halliwell and Gutteridge, 1991). Erythrocyte catalase is one of the enzyme systems, which protects hemoglobin and other cell components against oxidizing agents. It was demonstrated that catalase function becomes crucial in the red cell when additional sources of peroxides formation such as drug or ionizing radiation are present (Ben-Yosepeh and Shapira, 1973)

Many animal tumor cell lines are low in catalase activity; so many animal tumor cells are especially sensitive to H_2O_2 because of their low catalase activities. It usually protect against the DNA damage (Halliwell and Gutteridge, 1991).

2.2.4. Glutathione S- Transferase (GST):

Glutathione S-Transferase (GST) is a group of enzymes involved in the detoxification of numerous carcinogenic, mutagenic, toxic and pharmacologically active compounds (Mosialuo and Morgentern, 1990).

Microsomal Glutathione S-Transferase was incorporated into liposomes together with NADPH cytochrome P-450 reductase and cytochrome P-450 (Mosialuo, 1993). In order to show that Microsomal Glutathione S-Transferase can protect against lipid peroxidation, reconstituted systems were employed (Mosialuo, 1993). It is the main enzyme providing reducing equivalents to many cellular processes (Wittea *at al.,* 2005). It can also conjugate toxic products (4-hydroxynon-2-enal) of lipid peroxidation and thereby protect the cell. Report that the microsomal Glutathione S-Transferase can be activated by (4- hydroxynon-2-enal) (Mosialuo and Morgentern, 1989). The mechanism of up-regulating the enzyme activity during oxidative stress, finding that the activated enzyme increase its glutathione peroxidase

activity toward lipid hydro peroxides suggest that such up regulation would be beneficial during oxidative stress (Mosialuo and Morgentern, 1989). Exposure of lead chloride or lead acetate effect to the level of blood glutathione, Glutathione S-Transferase, peroxidase and reductase as well as glutathione regeneration process, also the blood was decreased glutathione concentration reaching a lowest value (Hunaiti *et al.*, 1999).

The activity of glutathione peroxidase activity and Glutathione S-Transferase, have the ability to reduce a series of phopholipid which can be formed during the peroxidation of biological membranes (Mosialuo, 1993). Antioxidant and antioxidant-related enzymes in the regulation of inflammatory mediators entail hierarchically arranged mechanisms ; (I) an antioxidant/pro oxidant signal is perceived in the context of modulating redox equilibrium; (II) when a pro oxidant signal prevails, an inflammatory response is augmented and, in the absence of an orchestrated antioxidant defense, cytokines may exacerbate the situation; (III) this delicate antioxidant/prooxidant equilibrium controls the routes that lead to the activation of downstream effectors, notably MAPKs, which in turn, along with upstream cofactors, signal the regulation of transcriptional entities; (IV) transcription factor regulation ensues to conceive a counteract, genetically-defined response in the face of oxidative challenge, with the prospects of limiting the debilitating effects of free radicals (Haddad and Harb, 2005).

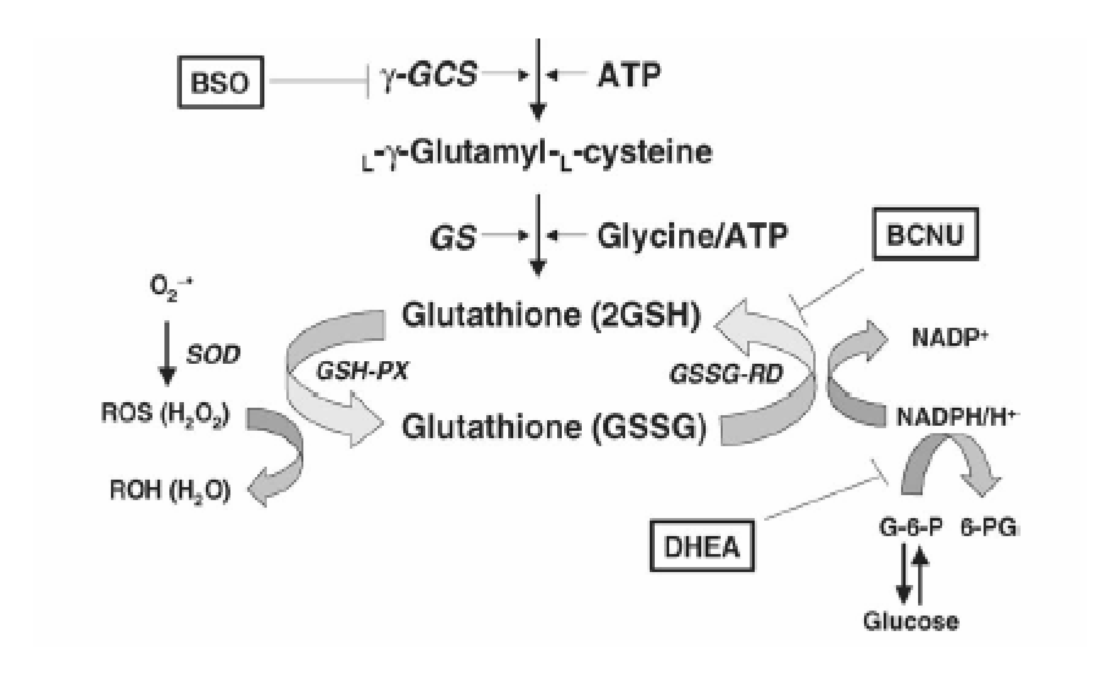


Figure 1.
The relationship between antioxidant enzymes
(Haddad and Harb, 2005)

2.3. Trace element supplementation

Trace elements are a small and yet extremely important part of grazing animals nutrition. Inadequate intake of any of the essential trace elements can result in reduced enzymes activities, more disease and decreased reproductive performance. Important trace elements in pasture systems are Co, Cu, Mn, Se and Zn (Underwood, 1981).

2.3.1. Methods of providing minerals to animals:

Indirect method of providing trace elements to grazing animal include use of mineral containing fertilizer, altering soil pH and encouraging growth of specific pasture species (McDowell, 1985). Underwood (1981) indicating that increasing soil pH influences forage mineral uptake, therapy causing deficiency of Cu and Co excesses of Se and Mo.

Whereas fertilizer treatment of the soil is an effective means of improving both the yield and mineral composition of herbage (McDowell, 1985). Increased mineral content of forage through fertilization has an additional advantage of assuring uniform mineral consumption, since all animals would be consuming higher quantities of minerals in the forage (Underwood, 1981). The major problem of free choice mineral supplements is that not all animals in herd will consume adequate quantities (McDowell, 1985). That recommended allowing for variation in the mineral requirement of individual animals. Conclude that animals have ability to consume minerals in amount needed to meat requirements, if a palatable mineral source is available (McDowell, 1985). Direct administration of minerals to animals in water, mineral licks, mixtures, and drenches, rumen preparations (i.e., Co pellets, Copper oxide needles, and glass bullets containing various trace elements), and injections are generally the most economical methods of supplementation (McDowell, 1985).

2.3.2. Factor affecting minerals consumption

The main factors that effect in minerals consumption are Soil fertility, forage type consumed, Salt content of drinking water and Palatability of mineral mixture (McDowell, 1985). Barrous (1977) reported that animals in native range consume more mineral supplement than improved pasture. Naturally high concentration of drinking water decrease supplement intake (McDowell, 1985). Denton (1970), noted that all mammals have the ability to taste salt, and there is a universal linking for salt. When animals are not allowed access to minerals for long periods of time, they may become so voracious that they often injure each other in attempting to reach salt, under

these conditions, they consume 2-10 times the normal daily quantities of minerals until their appetite is satisfied (McDowell, 1985).

Chapter Three
Design and methodology

3.1 Experimental sites

Three representative locations were selected in Al-Karak region. The locations were selected according to their availability of grazing sheep, rainfall and pasture. The three locations are: Aiy, Al Jedydeh and Al Qaser. The information regarding the location as follow:

3.1.1 Aiy:

It is in the South of Al- Karak region, it has green pasture, forage, trees, crops and grazing plants. In the spring season, Awassi sheep grazed on plant and forage that grow in this region. From late winter until summer. Sheep fed on crop residue, hay and cereals. In autumn and winter, animals fed on the cereals, hay, crop residue and dry grasses.

3.1.2 Al jedydeh:

It is in the east of Al karak region. It has a rainy winter and it has less pasture than other regions that have green pasture in spring season and early summer. In autumn and winter, the animals fed on cereals, hay and dry grasses.

3.1.3 Al qaser:

It is in the north of Al karak region. This has the same rainfall in winter to Aiy region, but has less grazing pasture than Aiy region. That animal's feeds in spring depend on green pasture. In summer they depend on crop residue, hay and cereals. In autumn and winter, the animals depend on hay, cereals and other crops residue.

3.2. Animals

A total of 45 grazing Awassi ewes, approximately same age and body weight, were randomly selected from three grazing herds (15 ewes from each location). All animals were ear tagged for identification for samples collection.

3.3. Samples collection:

Whole blood and serum samples were collected from jugular veins of each animal by using vacutainer tube with heparin and without heparin. Samples were collected four times, during year 2004/2005,

from each location according to the seasons and feeding calendar of Al-Karak region. The four seasons were: season I (spring) from April to May; season II (summer) from June to September; season III (autumn) from October to December and season IV (winter) January to march.

Blood samples tubes were placed in an angle of 45°C and transferred immediately to the laboratory and refrigerated at –20 C° until use for trace elements and enzyme activities assays.

Representative feed stuffs, pasture and soil samples were collected at the same time of bleeding from the 3 locations during the four feeding seasons. These samples were stored in clean plastic bags for further analysis.

Note: Equipments and Materials shows in Appendix II.

3.4. Blood sample preparation:

3.4.1. Whole blood preparations:

Blood samples were transferred immediately to the laboratory in ice box to avoid hemolysis. Blood samples were washed with 5 volumes of 0.9 % NaCl solution (9 gm/ L). Using centrifuge tubes for centrifugations of the blood samples, for 10 minutes at 3000 rpm (refrigerated centrifuge) and plasma was pipetted off. Washing repeated three times, in each of them plasma pipetted off and erythrocytes washed using repeated centrifugation. Then one ml of 0.9% NaCl was added to one ml of washed cells. 0.9 ml of distilled H_2O to 0.1 ml of hemolysated to take the 1:20 dilution of hemolysate. Then assay for enzymes activities were conducted or freezed in –20 ºC until time of analysis.

3.5. Blood serum preparation:

3.5.1. Serum separations:

Blood samples were centrifuged for 15 minutes at 3500 rpm. Separated serum was collected using Pasteur pipettes and transferred in serum sterilized plastic tubes, freezed (-20 ºC) until time of trace elements determinations.

3.5.2. Serum preparation for trace elements determination:

Using method described by AOAC (1990), Trichloroacetic acid (TCA) 10% were used for precipitated the proteins and collects the supernatants. The principles of the preparations depend on the TCA added 4 ml to 1 ml serum to give 5 X dilution, centrifuged for 15 minutes at 3000 rpm; tubes were clean and free of trace elements to avoid contaminations. The supernatants separated and placed in serum

sterilized plastic tubes. Freezing the supernatants until the time for assessments of Cu, Zn, Mn and Co by using Atomic Absorption Spectrophotometer (AAS).

Principles of Atomic Absorption Spectrophotometer (AAS):

Atomic absorption spectrophotometry flame (Varian. GTA 100 Spectra AA- 200). Resembles emission flame photometry in that a sample is aspirated into flame and atomized. The major difference is that flame photometry the amount of light emitted, whereas in Atomic absorption spectrophotometry alight beam is directed through the flame, into a mono chromator, and onto a detector that measures the amount of light absorbed by the atomized element in the flame. For many metals difficult to analyze by flame emission, atomic absorption exhibit superior sensitivity. Because each metal has its own characteristic absorption wavelength. A source lamp composed of that element is used; this makes the method relatively free from spectral or radiation interferences.

Analytical methods:

For analyzing trace elements levels, the atomic absorption spectro photometer was set to use the flame method. A copper hollow cathode lamp was used. This lamp source was set at a wavelength of 324.8 nm and the slit width was set at 0.5 nm for this analysis. The atomic absorption spectrophotometer was set to "PROMOT" for its measurements mode, "concentration" as its calibration mode, and "absorbance" as its instrument mode. For this particular analysis, the calibration algorithm was the new rational algorithm. The bulk standard concentration for (Cu) was set at 4.16 ppm and the standards were 2.0 ppm, 0.8 ppm and 0.4 ppm, for Zn was set at 2.0 ppm, and the standards were 1.2 ppm, 0.6 ppm and 0.2 ppm, for Mn was set at 2.0 ppm, and the standards were 1.2 ppm, 0.6 ppm and 0.2 ppm, and for Co was set at 4.0 ppm and the standards were 2.0 ppm, 0.8 ppm and 0.4 ppm.

The following setting were used for proper analysis of the samples: three second measurements, eight second pre-read delay, 6.0 mA lamp current, and the lamp was set in the "third lamp" position.

The serum size and the total sample weights were then entered into the appropriate computer program. To optimize the lamp, the flame was ignited and then the flame was extinguished. The flame was reigented, started and the pump tube was inserted into each sample.

3.6. Enzymes assay:

3.6.1. Glutathione peroxidase activity (GPx):

Reagents preparation:

The reaction mixture were composed of 0.2 M Tris-HCl buffer, pH 8.0, 1 U/ml glutathione reductase, 7 mM t-butylhydroperoxide and 2 mM NADPH. The activities of glutathione peroxidase were measured by following the rate of NADPH oxidation at 340 nm. The specific activity was expressed as U/ g hemoglobin.

Assay of GPx:

The activity of glutathione peroxidase was measured spectrophotometer-metrically using method modified by Hunaiti, *et al.* (1995). The assay mixture was contained 20 µl of 0.1 M GSH, 10 µl of 1:20 hemolysate, 100 µl of 1 U/ml glutathione reductase, 10 µl of 7 mM t-butylhydroperoxide, 760 µl of 0.2 M Tris-HCL buffer, and 100µl of 2 mM NADPH. The rate of NADPH oxidation from reduced form to oxidized form was followed at 340 nm.

3.6.2. Glutathione reductase activity (GR):

Reagent preparation:

The reagents consist of 0.033 M Oxidized glutathione, 50 mM sodium phosphate buffer pH 7.5 and 1:10 hemolysate.

Assay of GR:

The activity of glutathione reductase was measured in the hemolysate according to the method modified by Hunaiti, *et. al*. (1995). The assay mixture contained 100 µl of oxidized glutathione, 10 µl of 1:10 hemolysate, and 840 µl of 50 mM potassium phosphate buffer, pH 7.5, and 50 µl of 2 mM NADPH. The rate of NADPH oxidation was followed spectrophotometricaly at 340 nm. The specific activity was expressed as mU/ g hemoglobin.

3.6.3. Glutathiones S-transferases (GSTs):

Reagents preparation:

GST Buffer consists of 0.1 M potassium phosphate buffer pH 6.5, 0.1 M Glutathione reduced form (GSH), 100 mM EDTA, 1 mM HCL, and 0.1 M 1-Chloro-2,4-dinitrobenzene (CDNB).

Assays of GSTs:

The activity of glutathione S-transferase was measured in the hemolysate blood according to the modified method by Jakoby and Habig, (1980). The assay mixture contained 880 µl of GST- buffer, 50 µl of GSH, 50 µl of CDNB and 20 µl of 1:20 hemolysate blood; the specific activity was expressed as U/ g hemoglobin.

3.6.4. Catalase (CAT):

Reagents preparations:

The reagent consists of 50 mM potassium phosphate buffer pH 7.5 and 200 mM H_2O_2 (Hydrogen peroxide).

Assays of CAT:

The activities of catalase were measured spectrophotometrically using method described by Aebi (1984). The assays mixture contained 965 µl of 0.1 M phosphate buffer pH 6.5, 1mM EDTA, 50 µl H_2O_2 and 20 µl of 1: 20 hemolysate blood. The rate of oxidation of H_2O_2 to H_2O was followed at 240 nm. The specific activity was expressed as U/ g hemoglobin.

3.7. Soils sample assay:

Using extraction solution, which is efficient in calcareous soil. (Diethyline Tri Amine Penta Acetic Acid) (DTPA) A chelating agent was selected because it offered the most favorable combination of stability constant for the simultaneous Complexing of trace elements. The extraction is buffered at pH 7.30 which is a slightly alkaline range contains $CaCl_2$, so that equilibrium with $CaCO_3$ is established at a CO_2.

3.7.1. Reagent preparation:

DTPA extraction solution (PH 7.30) for 1-liter solution preparation; dissolve 1.976 g of DTPA, 14.92 g of TEA (CH_2-CH_2. BH_3) N, and 1.47 g of $CaCl_2.2H_2o$ in approximately 200 ml of H_2O, allow sufficient time for the DTPA to dissolve and diluted to approximately 0.9 L adjust the PH to 7.3 with 1:1 N HCL while stirring and diluted to 1 L. This solution is stable for several months.

3.7.2. Soils preparation:

The samples that collected throughout the year (12 samples) by the season from the three locations were preparations in the laboratory before trace elements determination by using atomic absorption spectrophotometer. Weight of 15 gm of fine soil, added to 30 ml of

DTPA solution that acted as a chelating agent for trace elements in the soils. Shaking then for 2 hrs with shaker. After 2 hrs, filtration the reagents using the filter papers and collected the suspensions. The suspensions then measured by atomic absorption spectrophotometer for Zn, Cu, Mn and Co.

The concentration of trace elements in soil were measure as ppm, the total mount of trace element in soil sample was calculated as the equation:
Amount of trace element= concentration absorb X (30 DTPA/15 g soil).

3.7.3. Plant samples assay:

Plant and supplements samples that collected were dried and classifieds, grinded by using (Minder). One gm of each sample was weight in crucible and ashing in muffle furnace at temperature 550-650 C ^{o} AOAC, (1990). After that added 2 ml conc. HCL in the samples in crucibles and placed for 2 hrs, then filtrated using filter paper in eyrnmayer flask 50 ml using 0.1 N HCL. Freezing the samples until assays for trace elements status in plant and supplements samples were performed using atomic absorptions spectrophotometer.

3.8. Statistical analysis:

The general linear model procedure of SPSS® version 11.0 was used to analyze the data for a complete randomized design with repeated measurements. The repeated measurements were seasons. The independent variables were season and location. The dependent variables were the concentrations of Cu, Zn, Mn, and Co in serum and the activities of Gpx, GR, GST, and CAT enzymes in whole blood. The Pearson correlation procedure of SPSS® was used to examine the correlation between the above trace elements and enzymes activities in blood of grazing ewes. Means were compared using the Scheffe test of SPSS®. Significance was declared at ($P<0.05$) unless otherwise noted.

Chapter Four
Finding and Discussion

Animals in many parts of Jordan consume diets that do not meet these exacting requirements. Adequate or marginal deficiencies or toxicities of this nature assume great importance in the nutrition and that affect the activities of antioxidant enzymes, some trace elements play a role in the activities of these enzymes. Gpx, as one of the primary antioxidant enzymes, is an important component in the protection against free radical damage to cells and thus is crucial to cell survival. Usually Gpx activity was considered as an indicator of selenium status in a variety of species. (Halliwell and Gutteridge, 1991).

The lack of adequate amounts of dietary nutrient may show a variation in trace elements concentration and enzymes activity throughout year in all locations. If ewes were fed additional trace elements during certain season, it can be possible to prevent disorders caused by deficiency of those elements in sheep maintained at pasture conditions.

The dry season is the most difficult time in the life cycle of grazing animal in southern part of Jordan, because of the poor quality and limited quantity of forges. During the dry season, animals usually supplemented with stored forages and crop residue that contains lesser amount of minerals. The Awassi sheep in southern part of Jordan were depend upon forages for most minerals and other nutrients requirements, because no mineral supplements are provided, the variations in mineral with seasons detected throughout the year, caused by variation in feed available according to seasons. In this study, our main objective was to investigate the possible effect of season on the concentration of trace elements and consequently the enzyme activities in grazing Awassi sheep on southern part of Jordan. Unfortunately, there are no adequately published regarding this topic in grazing sheep. According to the literature, very few studies were published regarding the trace mineral status of grazing sheep in Jordan. On the other hand, no studies investigated the enzymes activity and their change-according season.

In this work, we will discuss each location separately in term of trace elements status and enzymes activities. Moreover, differences between locations will be discussed for each season. The correlation between minerals level and enzymes activities will be investigated.

The adequate levels of trace elements in sheep blood serum, dietary requirements and soil content will be described. The adequate levels (i.e. levels believed to represent dietary sufficiency) of trace minerals in serum of sheep are: Cu (0.7- 2.0 µg/ml); Zn (0.8- 1.2 µg/ml); Mn (0.8- 5

µg/ml); (C0 0.2- 0.3 µg/ml) and Se (0.2 ng/ml) (Underwood, 1981 and Puls, 1988). In addition, both blood Gpx and blood or plasma Se concentrations give useful indication for Se nutrition (McDowell, 1985).

Moreover, the chemical composition of available feed (pasture and supplements) that fed the four seasons at southern part of Jordan shown in table 1, 2 and 3. The requirements levels of minerals in feed in this study are: Cu, 10 µg/g; Zn, 30 µg/g; Mn, 25 µg/g; and Co, 1 µg/g (ARC, 1980 and Underwood, 1981). In addition, the level of trace elements in soil at four seasons in southern part of Jordan in this study showed in table 4, 5 and 6. The critical levels of trace elements in soils as follow: Cu, 0.3 µg/g; Zn, 1.0 µg/g; Mn, 5.0 µg/g; Co, 0.1 µg/g and Se, 0.5 µg/g (Rhue and Kidder, 1983).

The means of trace element concentrations and enzyme activities in the three locations throughout the year are shown as a diagrams in appendix (I).

4.1. Effect of season on the concentration of Trace elements and enzymes activity in Aiy, Al- Jedydeh and Al-Qaser.

4.1.1. Effect of season on Aiy:

4.1.1.1. Trace elements:

The effects of season on the concentrations of Copper (Cu), Zinc (Zn), Manganese (Mn) and Cobalt (Co) in serum of grazing Awassi ewes in Aiy were reported in table 7.

There was no significant difference (P>0.05) in Cu concentration in blood serum of grazing Awassi ewes. Numerically, Cu concentration during season II (0.92 µg/ml) was higher compared with season I, III and IV (0.73, 0.87 and 0.85 µg/ml, respectively). Moreover, the concentration of Cu during season II, III and IV were fallen within adequate level, but marginal during season I.

Significant differences (P<0.001) in Zn concentration in blood serum of Awassi ewes was detected within seasons. Zinc concentrations in season I was significantly differ (P<0.05) than in season III and IV, but not compared with (P>0.05) levels during season II. The lower concentrations of Zn were found in season III (0.67 µg/ml) and VI (0.53 µg/ml), which considered marginal according to Puls, (1988).

Manganese concentrations in blood serum of grazing Awassi ewes were significantly differ (P>0.001) by season. Manganese concentration in season I was significantly differ (P<0.001) than season III, but no differences were reported when compared with season II and IV. Moreover, the value of Mn concentration was deficient throughout the

year. The highest value was detected during season III (0.25 µg/ml), according to Puls, (1988).

A significant difference ($P<0.05$) in Co concentrations in blood serum of grazing Awassi ewes was detected within season I and II. In addition, the concentrations of Co in blood serum of ewes during season III and IV were below detection limit, which indicated high deficiency during these two seasons.

Pastrana and other (1991), reported a high Cu concentration in blood serum of grazing sheep during the dry season, but not effected ($P> 0.05$) by season. This result agreed with our finding. Abdelrahman (2003) reported from a study conducted in the northern part of Jordan using grazing sheep, serum Cu concentration was in adequate level (1.48 and 1.50 µg/ml), and there was no effect of season on the Cu concentration, which disagree with our finding. In this study Cu concentration in the blood serum was lower than reported by Abdelrahman, (2003). Moreover, Ogawang (1988) reported a change in Cu levels by season which is higher in summer and low in winter. This conflict in findings may cause as a result of low feeding and feeds available, Cu concentrations, amount consumed and physiological conditions of animals. The low Cu concentration in feed and soil (Table 1 and 4) during spring season (I) (0.73 µg/ml) that agreed with Albel and others (1979) findings, who reported a possibility of Cu deficiency, especially during the green season, they explained this result by assuming a presence of high level of Mo in the available feedstuffs.

Yokus and others (2004) supported our finding by reporting a significant change in Zn concentrations by season for grazing sheep throughout year. Moreover, Abdelrahman (2003) reported that no significant effect of season on Zn concentration in blood serum of grazing sheep in northern Jordan. In addition, Zn concentrations were considered in the deficient margin which disagreed with our findings. In this study blood serum Zn concentration in season I and II were in adequate level (0.90 and 0.86 µg/ml, respectively). These results agreed with Albel and other, (1979) who reported adequate level of Zn concentration throughout the year, White and other (1995), they reported an adequate level in Zn concentration during spring season, but below during other seasons. The variation in feed available throughout year were detected may affect Zn concentration in serum of ewes, and the low feed available during dry season may effect on Zn level in serum during these season. Pastrana and other (1991), reported that Zn concentrations tended to be higher during the rainy season.

In this study, Mn concentrations in blood serum of grazing ewes was significantly affected by season and deficient throughout year. This agreed with Abdelrahman (2003) who reported Mn deficiencies levels in blood serum of grazing sheep at northern Jordan. Moreover, Albel and other (1979) reported the same trend by detecting Mn deficiency level in liver sheep throughout the year. The low levels of Mn in serum may be due the low level of Mn in feed and soil, due to relationship between Mn level in soil and Mn intake by animals (Sutmoller *et al.*, 1966).

Abdelrahman (2003) reported an adequate Co level in blood serum of grazing sheep throughout the year in northern part of Jordan. In addition, he reported a significant effect of season on the Co concentration. Our findings disagree with Abdelrahman (2003) because Co level in blood serum of the ewes in season III and IV were below the detection limits, which considered to be highly deficient. In addition, no significant effect of seasons was detected. This also disagreed with findings of Yokus and other (2004). The low Co value in these seasons may be due to a variation in feed available due to low feed available and soil, were a number of world locations have reported this conditions, Co deficiency in soil (McDowell, 1981).

In Aiy, Cu concentrations in the blood serum of Awassi ewes were adequate throughout the year. Zinc were adequate only during season I and II, but marginal during the other seasons. Moreover, Mn and Co were deficient throughout the year.

4.1.1.2. Activity of enzymes:

Table 8. Shows the effect of season on the activities of Glutathione peroxidase (Gpx), Glutathione reductase (GR), Glutathione-S transferase (GST) and Catalase (CAT) enzyme in whole blood of Awassi ewes in Aiy.

No significant differences (P>0.05) in the activity of Gpx enzyme in whole blood of Awassi ewes from season to another, but numerically the high activities reported in season II and III (18.14 and 18.24 U/g Hb, respectively).

Significant variation (P<0.01) was observed in the activity of GR within different season. No significant difference (P>0.05) was found between season I and II, but GR activity during both season was significantly differ (P<0.05) when compared with the GR activity during season III and IV. Moreover, the GR activity during season I and II was

very low (775.5 and 724.4 mU/ g Hb, respectively), and very high during season III (1801.8, 1394,8 mU/ g Hb, respectively).

Season showed a significant effect (P<0.001) in the GST enzyme activities in whole blood of Awassi ewes. The GST activities in blood of ewes during season I was significantly differ (P<0.001) than other seasons. No significant difference was detected during season II and IV. Moreover, GST activities in blood of Awassi ewes during season III was the highest (1.83 U/g Hb) and significantly differ (P<0.001) from the activity in the seasons I, II and IV (1.05, 0.64 and 0.62 U/ g Hb, respectively).

A significant difference (P<0.001) was detected in activity of CAT in whole blood of Awassi ewes within different seasons. The CAT activity during seasons I was the highest (3.70 U/ g Hb) and significantly differ (P<0.001) from season II and III (1.8 and 2.05 U/g Hb, respectively), but not significantly differ (P>0.05) compared with season IV (2.83 U/g Hb).

We demonstrated that ewe's sheep erythrocytes Gpx activity showed no change during the four seasons, whereas the CAT, GR and GST activities change with season.

These finding would be explained by less variation in Cu concentration during the four seasons. No change in Gpx activity during the season was found. This may be, due to the activity of other enzyme associated with oxidant such as GST against lipid peroxide as a result of lipid oxidation by Cu and other oxidants, or the animals were less exposed to oxidant during the four seasons.

The Se status of animals is indirectly measured by the assay; Se-dependent Gpx enzyme (Paglie and valentie, 1967). The lowest activity of Gpx was in spring season. This agrees with previous study, since Selenium nutrition varies throughout the season with the lowest level in wet season (ARC, 1980). Serum Se concentrations are directly related to dietary Se intake in many animal species (Pamukcu *et al.*, 2000). Therefore, a variation in Se level affects the activity of Gpx, were low activities finding in spring and winter season, but Se was at normal level during other seasons.

Few studies examined these antioxidant enzyme activities during the four seasons. It has been suggested that antioxidant enzyme activities e.g. CAT in human erythrocytes are influenced by ageing (McElroy *et al*., 1992).

Some researcher have shown that GPX and CAT activity decreased and increased MDA level in the blood of hypertension patients (Redon *et al.*, 2003) and an observed increment GST activities, which substitute

for Gpx activity in condition where Gpx activity is insufficient for the reduction of organic peroxide (Loguercio *et al*., 1996). Moreover, others showed decreased Se levels, was connected with decreased GPx activity (Berger *et al*., 1995). Therefore, increase activity of Se-independent GST in conditions of lipid peroxidation process.

It has been reported that CAT activity was lower in iron-deficient children than in normal. (Macdougall, 1972). Kasperczyk and other (2004), showed lower activity of GPx in the persons exposed to heavy metals (Pb), and be also as the result of insufficient increase of GR, which supplies reduced glutathione for GPx.

It seems that the tendency to increase is due to a rise in the requirement for GR, which is necessary to correct the function of GPx and GST (Kasperczyk *et al*., 2004). The role of trace elements has been described recently. Among these, Zn and Cu are essential trace elements and function as co-factors of antioxidant enzymes.

In Aiy, as a conclusion, high activity of Gpx was during season II and III, whereas high GR activity was in season III. GST and CAT activities were during season I and III. A significant difference in GR, GST and CAT by season was detected, but no significant differences in Gpx activity by season was observed.

4.1.2. Effect of seasons on Al-Jedydeh

4.1.2.1. Trace elements

The effects of season in the concentrations of Cu, Zn, Mn, and Co in blood serum of the Awassi ewes in Al-Jedydeh location were reported in table 9. There was significant difference ($P<0.001$) in Cu concentration in blood serum of Awassi ewes was detected within seasons. Cu concentration in season I was significantly differ ($P<0.001$) than in season II, but not compared with ($P>0.05$) than in season III and IV. Numerically, Cu concentration during season II (1.12 µg/ml) was higher compared with season I, III and IV (0.93, 0.70 and 0.97 µg/ml, respectively). Moreover, the concentration of Cu during seasons I, II and IV were fallen within adequate level, but marginal during season III.

Significant difference ($P<0.001$) in Zn concentration in the blood serum of grazing Awassi ewes was detected within seasons. Zinc concentration in season I was Significantly differ ($P<0.001$) than in other seasons. Zinc concentration in season II was significantly differ ($P<0. 001$) than in other seasons, but not compared with ($P>0.05$) levels during season III with season IV detected. The higher concentrations

of Zn were found in season I and II (0.95 μg/ml, 1.2 μg/ml, respectively), which considered in adequate level, but marginal during season III and IV (0.74, 0.66 μg/ml respectively).

Manganese concentrations in blood serum of grazing Awassi ewes was significantly differ (P<0.001) by seasons. Manganese concentrations in season I, II and IV were significantly differ (P<0.001) than in season IV, but no differences reported when compared with Season III. Moreover, the value of Mn was deficient throughout year. The highest values were detected in season III and IV (0.16 μg/ml, and .18 μg/ml, respectively).

No significant difference (P>0.05) in Co concentration in blood serum of Awassi ewes was detected within season, In addition, the concentrations of Co in blood serum of ewes during seasons III and IV below the detection limit, which indicated the high deficiency during these two seasons. Moreover, Co deficient showed throughout the year.

Abdelrahman (2003) reported an adequate level in the serum throughout the year. Which mean a significant of effect of season on Cu concentration in the blood of Awassi sheep. This in agrees with our finding. On the other hand, Yokus and other; (2004) disagreed with our finding. Moreover, the seasonal variation was also reported by Abdelrahman and other (1998) in Cu concentration in blood serum of grazing cattle which also supporting our finding. In addition, Pastrana and others (1991) supported our finding by reporting Cu concentration were deficiency during rainy season than other seasons.

A significant change in Zn concentrations by season for grazing sheep throughout year (Yokus *et al.*, 2004) was reported. The level of Zn during spring and summer seasons in adequate our finding agreed with results by White *et al.*, (1995) who reported an adequate level in spring season and below an adequate level in other seasons, that the same trend in Aiy location, an adequate level in season I and II but marginal in season III and IV according to Puls (1988). Abdelrahman (2003) reported low Zn concentration which an adequate level in serum of sheep in northern Jordan throughout the year. The result agree in season III and IV (0.74 and 0.66 μg/ml respectively), the low Zn levels of feed and soil (Table 2 and 5) may be effect on the level of Zn in blood serum especially in season III and IV.

The low of Mn level during season I and II in Al-Jedydeh location as the same in Aiy location observed, completely agreed with Albel *et al.*, (1979). This differences may be caused by complete repulsion Mn throughout seasons III and IV from overall feedstuff, and may be the

type of feed and soil have deficiency in Mn level during season I and II. Sutmoller and other (1966) reported a relationship between Mn of soils and Mn intake by animals.

A deficiency in Co concentration throughout the year found in Al-Jedydeh location, the result disagree with our finding by Abdelrahman (2003) who reported a significant effect of season in Co concentration and an adequate Co level in blood serum of grazing sheep in northern part of Jordan. This trend was the same in Aiy location for season III and IV, which below the detection limit. This might be the low Co intake in these seasons because variation in feed composed available and Co soil component.

In Al-Jedydeh, Season caused a significant effect on Cu, Zn and Mn concentrations in blood serum of grazing Awassi ewes, but not for Mn and Co. Zinc concentration was marginal during seasons III and IV, but Co were deficient throughout the year.

4.1.2.2. Activity of enzymes:

Table 10. Shows the effect of season on the activities of Glutathione peroxidase (Gpx), Glutathione reductase (GR), Glutathione-S transferase (GST) and Catalase (CAT) enzyme in whole blood of Awassi ewes in Al-Jedydeh.

A significant difference (P<0.05) in Gpx activity was found in Awassi ewes within different seasons. The Gpx activities during season III were the lowest (12.04 U/ g Hb) and significantly differed (P<0. 05) from season II and IV (22.80 and 23.01 U/g Hb respectively), but in season I (19.70 U/ g Hb) no significant difference (P>0.05) was found compared with other seasons.

Significant variation (P<0.001) was observed in the activity of GR within seasons. No significant differences (P>0.05) found between season I and II, but GR activity were significantly differ during both seasons (P<0.001) when compare with season III and IV. Moreover, the GR activity during season I and II were very low (542.83 and 660.00 U/g Hb, respectively), and very high during season III and IV (2890.03, 1110.92 mU/g Hb, respectively).

A significant effect (P<0.01) in the GST activities in whole blood of Awassi ewes was detected during seasons. The activities of GSTs during season III were significantly differ (P<0.01) than other seasons. No significant differences (P>0.05) were detected during seasons I, II and IV. Moreover, GST activities during season III were the highest (1.83 U/g Hb).

No significant difference ($P>.05$) was found in the activity of CAT in whole blood of Awassi ewes from season to another, but numerically the highest activities reported in season II (2.44 U/g Hb).

Significant change in Gpx ($P<0.01$), GR and GST activities during the seasons were found, might be explained by significant variation in Cu, Zn and Mn concentration during the four seasons or the animals would be exposed to oxidant during the four seasons.

We demonstrated that ewe's sheep erythrocytes Gpx activity showed variation during the four seasons, this is because of the increase level of Cu and Zn concentrations. These two metals were considered as a cofactor for the SOD enzyme, which worked in parallel with the Gpx (Paglie and valentie, 1967).

The lowest activities of Gpx were in spring and autumn. This agrees with previous study, since Selenium nutrition varies throughout the season with the lowest level in wet season (ARC, 1980). Therefore, a variation in Se level affects the activity of Gpx. This indirectly indicated the variation of Se levels within the seasons. Kasperczyk and other (2004), showed lower activity of GPx in the person exposed to heavy metals (Pb).

Redon and others reported in previous work that the level of Gpx and CAT activity in the blood of hypertension patients decreased and were associated with increased MDA level and an observed GST activities increment. Gpx activity sometimes substitutes for CAT in condition where Gpx activity is sufficient for the reduction of organic peroxide.

The CAT activity was not changed with season. This may be due to the type of oxidant associated with lipid peroxide other than the hydrogen peroxide. Macdougall (1972) has been reported that CAT activity was lower in iron-deficient children than in normal. High activities of GR during dry season may be due to the nature of this region, which showed high dusts level during summer and autumn, which may have heavy metal, that cause high activities of GR or due to the type of feed supplement during these season.

In Al- Jedydeh, high activity of Gpx during season II and IV, high activity for GR and GST during season III and high activity of CAT during seasons II and III. Significant differences by season in Gpx, GR and GST activities, but no significant differences in CAT activity by season were detected.

4.1.3. Effect of season in Al- Qaser:

4.1.3.1. Trace elements:

The effect of season in the concentrations of Copper (Cu), Zinc (Zn), Manganese (Mn), and Cobalt (Co) in the serum of grazing Awassi ewes in Al-Qaser reported in table 11. There were a significant differences (P<0. 01) in Cu concentration in blood serum of grazing ewes were detected within seasons. Copper concentration in season I was significantly differ (P<. 01) than in season II, but not compared with (P>0.05) levels during season III and IV. Moreover, the concentration of Cu during seasons I and IV (0.87 and 0.84µg/ml, respectively) was fallen within adequate level, but marginal in season II and III (0.58 and 0.76 µg/ml, respectively).

A significant difference (P<0. 01) in Zn concentration in blood serum of Awassi ewes was detected within seasons. No significant differences (P>0.05) were found between season I and II, but Zn concentration during both seasons were significantly differs (P<0.01) when compare with Zn concentration during season III and IV. Moreover, the Zn concentrations during season I and II were adequate (0.95 and 1.08 µg/ml respectively), and were marginal during season III and IV (0.66 and 0.62 µg/ml respectively).

Manganese concentrations in blood serum of Awassi ewes were significantly differ (P<0.001) by season. Manganese concentrations in season I and II were significantly differ (P<0.001) than in seasons III and IV, but no differences reported when compared between season II and I. Moreover, the lower value in Mn concentration seasons I and II (0.03 and 0.03 µg/ml, respectively). The highest value during season III (0.23 µg/ml). All values were considered deficient throughout year.

No significant difference (P>0.05) in Co concentration in blood serum of Awassi ewes was detected. In addition, the concentration of Co in blood serum of grazing ewes during season III and IV were below detection limit, which indicated the high deficiency during these two seasons.

Yokus and other (2004) supported our finding by reporting seasonal variations in serum blood Cu concentrations. Adequate levels detected in all season except season II that was marginal level (0.58 µg/ml). Albel *et al.,*(1979) reported that low level of Cu concentration during all seasons. Copper deficiency is often caused by Mo and S interfering with Cu utilizations (Underwood, 1981) or may be due to low feed available. Moreover, the low Cu concentrations in both soil and feed may cause low level of Cu in blood serum in specific season (Table 3 and 6). The significant differences in Cu concentration throughout the year may be the

variation in feed and soil throughout the year. Moreover, White *et al.,*(1995) reported an adequate level of Zn in liver of Awassi sheep in northern Jordan in spring season, but low in other seasons. Pastrana and other (1991), reported in a study concluded using sheep the only trace mineral affected by season was Zn, which had a lower value during the dry season, and this agree with our finding and Abdelrahman, (2003) reported a below an adequate level in Zn concentration in serum of sheep throughout year in season III and IV (0.66 and 0.62 μg/ml, respectively). In dry season lack of Zn level in feed and soil may affect the level of Zn in sheep. Moreover, Mn deficiency was detected, our finding completely agreed with result of Abdelrahman, (2003) who reported a significant effect of season in Mn level in blood serum of sheep, Albel *et al.,* (1979) reported low level of Mn in liver and serum of grazing Awassi sheep throughout year.

Cobalt in season III and IV were below the detection limit, may be the low Co value in these seasons. Our finding disagree with the result of Abdelrahman (2003) who reported a significant effect of season and adequate Co level in blood serum of grazing sheep in northern part of Jordan throughout the year. A high number of world regions have reported deficiency in Co concentration (McDowell, 1981).

In Al-Qaser, season caused a significant effect on Cu, Zn and Mn concentrations in blood serum of grazing ewes, but not for Co. Copper deficiency were during season II, Zn during season III and IV. Mn and Co deficient throughout the year were showed.

4.1.3.2. Activity of enzymes:

The effect of season on the activity of Gpx, GR, GST and CAT enzyme in whole blood of Awassi ewes in Al-Qaser was shown in table 12.

A significant difference ($P<0.05$) in the activity of Gpx within seasons was detected. No significant difference ($P<0.05$) were found between season I and III, but Gpx activity during both seasons were significantly differ ($P<0.05$) when compare with the Gpx activity during season II and IV. Moreover, the highest activity of Gpx was in season II and IV (19.6 U/g Hb, 20.39 U/g Hb, respectively), and the lowest was during season I (13.30 U/g Hb).

A significant variation ($P<0.001$) in the GR activity in whole blood of Awassi ewes within different seasons was detected. No differences ($P>0.05$) were found between I, II and IV, but GR activities during these seasons were significantly differ ($P<0.001$) when compare with III. Moreover, the GR activity during season I and II was very low, (752.97

and 734.70 mU/g Hb, respectively), and very high during seasons III and IV (2085.2 and1022.10 mU/g Hb, respectively).

Season showed a significant difference (P<0.001) in the GST enzyme activity in whole blood of Awassi ewes. The GST activities in whole blood of Awassi ewes during season III were significantly differ (P<0.001) than other seasons. No significant differences (P>0.05) were detected during season I, II and IV. Moreover, GST activities in whole blood of ewes during season III were the highest (2.04 U/g Hb) but lower in season I, II and IV (0.88, 0.98 and 1.15 U/g Hb, respectively).

A significant difference (P<0.01) was detected in activity of CAT in whole blood of Awassi ewes within seasons was observed. The CAT activities during season III were the lowest (1.29 U/g Hb) and significantly differ (P<0.001) from season I, II and IV (2.41, 2.74 and 2.38 U/g Hb, respectively).

Our finding would be explained by variation (P<0.01) in Cu, Zn and Mn concentration by season. Significant change in Gpx (P<0.05), GR, GST and CAT activities during the season were found. As far as the activities of these enzymes are associated with oxidative stress which is a result of exposure of the animal to oxidant such as metals or due to physiological, age and seasonal variation and due to the type of feeding during the four seasons.

The trace elements Fe, Cu, zinc, Zn and Se are essential components of major antioxidant enzymes in blood such as CAT, Cu/Zn-SOD, Gpx, GR and GSTs.

The result of insufficient Cu, Zn and Mn during the four seasons, will effect on the antioxidant enzyme activities, since these are essential components of major antioxidant enzymes in blood. This relationship was shown in figure 1. High activities of these enzymes during dry season may be due to the nature of this region, which showed high dusts level during summer and autumn, which may have heavy metal,

In Al- Qaser, high activity of Gpx during season II and IV, high activity for GR and GST during season III and high activity of CAT during seasons I and IV were detected.

4.2. Effect of location on the Trace elements concentration and enzymes activity by season in Aiy, Al- Jedydeh and Al- Qaser:

4.2.1. Effect of location during season I:

4.2.1.1. Trace elements:

The effect of locations in the concentrations of Copper (Cu), Zinc (Zn), Manganese (Mn), and Cobalt (Co) in the serum of the Awassi ewes in season I reported in table 13.

There were no significant differences (P>0.05) in Cu concentration in blood serum of Awassi ewes. Numerically, Cu concentration in Aiy (0.83 µg/ml) was lower compared with other locations. Moreover, the Cu concentration in Aiy, Al-Jedydeh, and Al-Qaser fallen within adequate level.

No significant differences (P>0.05) in Zn concentration in blood serum of Awassi ewes within locations. Numerically, the concentration of Zn in Al-Qaser and Al-Jedydeh locations (0.90, 0.87 and 0.83 µg/ml, respectively) were higher compared with other seasons. Moreover, the Zn concentration in three locations fallen within adequate level.

No significant difference (P>0.05) in Mn concentration in blood serum of Awassi ewes within locations. Numerically, the Mn concentration in Al-Jedydeh (0.07 µg/ml) was higher compared with other locations. Moreover, the value of Mn concentration was considered deficiency during season I.

A significant difference (P<0.001) in Co concentration in blood serum of Awassi ewes were detected within Al-Qaser, but no differences (P>0.05) found between Aiy and Al-Jedydeh. In addition, the Co concentrations in blood serum of Awassi ewes in adequate level during season I. Moreover the highest value in Al-Qaser (0.15µg/ml).

Copper concentration during season I were found below adequate level (0.73 µg/ml), Albel and other supported our finding by reporting a below level of Cu concentration in sheep during rainy season due to high level of Mo in feed. High intake of Mo, which often occur in pastures based diets, especially in winter and spring season, reduces Cu intake by the animals (Galloway *et al,* 2000). No effect of location was found, and no study regards our finding available.

Yokus and others (2004) reported that no effect of season in the level of Zn in sheep, this result agreed with our finding. Adequate level in Zn concentration in serum of sheep in locations in season I (spring) was detected, feed available may effect on Zn concentration in serum of sheep. In spring season, feed more available than other seasons. Serum Zn is a reasonable status criterion; however, values are susceptible to animal stress during sampling and can fluctuate rapidly (Underwood, 1981). In addition, Abdelrahman (2003) who reported deficiency in Mn

in blood serum of grazing Awassi sheep during spring season, no significant effect of location in Mn level in spring season, but no study available regarding our finding.

Pastrana and other (1991), reported in study of sheep, Liver Co concentration was in adequate level during rainy season, this result disagree with our finding. Abdelrahman, (2003) reported an adequate Co level in serum sheep in northern part of Jordan, the lowest Co level in Aiy and Al-Jedydeh locations, which may be the low Co value in these locations and lack variation in feed available due to low feed available and soil. Numbers of world locations have reported these conditions (McDowell, 1981).

In season I, location didn't show any significant differences on Cu, Zn and Mn concentrations, but only Co with high value in Al-Qaser.

4.2.1.2. Enzymes activity:

The effect of location on the activities of Gpx, GR, GSTs and CAT enzymes in whole blood of Awassi ewes in season I was shown in Table 14.

No significant difference ($P>0.05$) in the activity of Gpx enzyme in whole blood of Awassi ewes from location to another was detected, but numerically the high activity reported in Al-Jedydeh (19.70 U/ g Hb) and the lowest activity was Al-Qaser (13.30 U/g Hb).

A significant variation ($P<0.01$) in the activity of GR within different locations was detected. No significant differences ($P>0.05$) were found between Al-Jedydeh and Al-Qaser, but significantly differ when compared with Aiy location ($P<0.01$). Moreover, the GR activity was high in Aiy and Al-Qaser (775.50, 752.97 mU/g Hb, respectively) and low in Al- Jedydeh (542.83 mU/g Hb).

No significant effect ($P>0.05$) in the GST activity in whole blood of Awassi ewes was observed in season I. Moreover, numerically, the highest activity of GST in Al-Jedydeh was found, compared with other locations (1.14 U/g Hb).

A significant difference ($P<0.001$) was detected in activity of CAT in whole blood of Awassi ewes within locations. The CAT activity in Aiy was the highest (3.70 U/g Hb) and significantly differ ($P<0.001$) from Al-Jedydeh and Al-Qaser (1.80 and 2.41 U/g Hb, respectively), but no significant differences ($P>0.05$) between Al-Jedydeh and Al-Qaser were detected

The results agree with many studies, reported a deficiency in Se in grazing animals during wet seasons (ARC, 1980), which reflect low

activities of Gpx enzyme. So a variation in Se level between locations reflects differences in the activity of Gpx within locations.

No change in Gpx (P>0.05) activity between locations during season I was found. This may be, due to the activity of other enzyme associated with oxidant such as GST against lipid peroxide as a result of lipid oxidation by Cu and other oxidants.

Some researchers have shown that GPX and CAT activity had positive correlation with MDA level in the blood (Redon *et al.*, 2003). This agrees with our result. Moreover, there was a positive correlation observed between GST and GR activities in season I (Macdougall, 1972).

In season I, high activity of Gpx and GST were showed in Al-Jedydeh, and high activity of GR and CAT in Aiy and Al- Qaser. Significant differences in GR and CAT activities within locations were detected, but no significant differences in activities of Gpx and GST observed.

4.2.2. Effect of location during season II:

4.2.2.1 Trace elements:

The effect of locations on the concentrations of Copper (Cu), Zinc (Zn), Manganese (Mn), and Cobalt (Co) in the serum of the Awassi sheep in season II presented in table 15.

There was a significant difference (P<0.001) in Cu concentration in blood serum of grazing Awassi ewes within locations. No significant differences (P>0.05) were found between Aiy and Al-Jedydeh, but Cu concentrations in Al-Qaser were significantly differ (P<0.001) when compared with other locations. Moreover, numerically Cu concentration was higher in Al-Jedydeh and Aiy (1.12 µg/ml and 0.92 µg/ml, respectively), than Al-Qaser (0.58 µg/ml). Moreover, Cu concentration fallen within adequate level in Aiy and Al-Jedydeh, but deficiency in Al-Qaser.

A significant difference (P<0.01) in Zn concentration in blood serum of Awassi ewes was detected within locations. Zn concentration in Al-Jedydeh location was significantly differ (P<0.01) than in Aiy location and Al-Jedydeh, but not significantly differ (P>0.05) compared with other locations. Moreover, numerically the high level of Zn reported in Al-Jedydeh (1.20 µg/ml), but the lowest level in Aiy (0.86 µg/ml), the concentrations of Zn in three locations fallen in adequate level.

Manganese concentration in blood serum of grazing ewes was significantly differ (P<0.05) by location. Manganese concentration in Al-Qaser was significantly differ (P<0.05) than in Aiy and Al-Jedydeh,

but no differences (P>0.05) in other locations reported. Moreover, the concentration of Mn had fallen within deficient level in all locations.

A significant differences (P<0.001) in Co concentration in blood serum of Awassi ewes were detected within Al-Qaser, but no differences reported when compared Aiy and Al-Jedydeh. Moreover, the lowest value of Co in Aiy and Al-Jedydeh (0.08 and 0.07µg/ml, respectively), the highest values in Al-Qaser (0.13µg/ml) that in adequate level.

Adequate level of Cu concentration in blood serum of Awassi ewes detected during season II, our result agreed with Abdelrahman, (2003) reported adequate level of Cu concentration in serum of Awassi sheep throughout year. Most authors determined that the highest serum Cu measurements were in the summer (Yokus *et al.*, 2004), due to variations in available feedstuffs for grazing animal in Southern part of Jordan and low Cu concentration of soil in A-Qaser. The low Cu concentration in Al-Qaser was found (0.58 µg/ml).

Adequate level in Zn concentration in blood serum of Awassi ewes in all locations in season II was detected, this result disagrees with our finding of Abdelrahman (2003). Moreover, Albel and other (1979) reported low level of Zn in blood serum of sheep during summer season. This result supported our results finding. Moreover, Mn level in feed and soil reflect Mn level in animal (McDowell 1981), significant effect of location in Mn concentration in grazing ewes found, our but no study supported our finding.

Abdelrahman (2003) and Pastrana and other (1991), reported an adequate level of Co concentration during summer season. These results disagree with our finding. Lacks of Co level in feed available soil were a number of world locations have reported this conditions (McDowell, 1981).

In season II, Cu, Zn, Mn and Co were differ by location, Mn and Co were deficient in the three locations.

4.2.2.2. Enzymes activity:

Table 16. Shows the effect of location on the activities of Gpx, GR, GST and CAT enzymes in whole blood of Awassi ewes in season II. A significant difference (P<0.01) in the activity of Gpx enzyme in whole blood of Awassi ewes within different locations was detected. No significant differences (P>0.05) were found between Aiy and other locations, but Gpx activity in Al-Jedydeh and Al-Qaser were significantly differ (P<0.01). Moreover, numerically the highest activity

of Gpx in Al-Jedydeh (22.80 U/ g Hb) and in Aiy and Al-Qaser (18.14 and 19.60 U/ g Hb) activity was at normal level.

No significant difference (P>0.05) in the activity of GR enzyme in whole blood of Awassi ewes within different locations was detected, but numerically the lowest activity reported in Al-Jedydeh (660.0 mU/g Hb).

A significant difference (P<0.05) in the GST activity in whole blood of Awassi ewes was detected. The GST activity in blood of ewes in Al-Jedydeh location was significantly differ (P<0.05) with other locations. No significant difference (P>0.05) was detected in Aiy and Al-Qaser. Moreover, GST activity in blood of ewes in Al-Jedydeh was highest (1.28 U/g Hb) compared with Aiy and Al-Jedydeh (0.64 and 0.98 U/ g Hb).

No significant difference (P>0.05) was detected in the activity of CAT in whole blood of Awassi ewes within different locations. Moreover, the activity of CAT highest in Al-Jedydeh and Al-Qaser (2.44, 2.47 U/ g Hb respectively) compared with Aiy.

The results agree with many studies, White *et al.*, (1995) reported high level of Se in grazing animal in northern part of Jordan, that reflect high activities of Gpx enzymes. But a variation detected between locations, may caused by a differences in feed available. So a variation in Se level between locations reflects differences in the activity of Gpx within locations.

We demonstrated that ewe's sheep erythrocytes GR and CAT activities showed no change between locations, whereas the Gpx and GST activities change with season.

Kasperczyk and other (2004), showed lower activity of GPx in the person exposed to heavy metals (Pb), and be also as the result of insufficient increase of GR, which supplies reduced glutathione for GPx. High activities of GR during summer season may be due to the nature of this region, which showed high dusts level during summer and autumn, which may have heavy metal, that cause high activities of GR during season II.

In season II, high activity of Gpx and GST in Al-Jedydeh was showed, and the high activity of GR and CAT in Al-Jedydeh and Al-Qaser. A significant difference between location in activities of Gpx and GST, but no significant differences in activities of GR and CAT observed.

4.2.3. Effect of location during season III:

4.2.3.1. Trace elements:

The effects of locations on the concentrations of Copper (Cu), Zinc (Zn), Manganese (Mn), and Cobalt (Co) in the serum of the Awassi ewes in season III were reported in table 17.

They were no significant differences (P>0.05) in Cu concentration in blood serum of Awassi ewes. Numerically Cu concentration in Aiy location (0.87 µg/ml) higher compared to Al-Jedydeh and Al-Qaser (0.70 and 0.76 µg/ml, respectively). Moreover, no significant differences (P>0.05) in Zn concentration in blood serum of Awassi ewes were within locations. Moreover, numerically the concentration of Zn had fallen within marginal level in Aiy, Al-Jedydeh and Al-Qaser (0.67, 0.74 and 0.66µg/ml, respectively).

Manganese concentrations in blood serum of Awassi ewes were not significantly differ (P>0.05) by locations. Moreover, numerically, the concentration of Mn in blood serum of Awassi ewes fallen a deficiency in Aiy, Al-Jedydeh and Al-Qaser (0.25, 0.16 and 0.23 µg/ml, respectively).

The concentration of Co in season III were not detected in all locations, were below detection limit, which indicated the high deficiency during these season.

Copper concentration was below adequate level throughout 2 locations (Al- Jedydeh and Al-Qaser). This deficiency was observed in a similar manner between the locations in both seasons (Erdogan *et al.,* 2004). Moreover, Cu deficiency in liver sheep throughout year reported in northern Jordan (Albel *et al.*, 1979), but disagreed with our result and finding by Abdelrahman (2003) who reported adequate level of Cu throughout year in northern Jordan.

A lower value of Zn level in grazing sheep during the dry season was detected (Pastrana *et al.,* 1991). This result agreed with our finding, but disagrees with result by White and other (1995). They reported an adequate level in autumn and winter seasons in blood serum of sheep. Serum Zn is a reasonable status criterion (Underwood, 1981). Feed available may effect on Zn concentration in serum of sheep. Lack of feed available may affect the level of Zn in blood serum of Awassi ewes in all location in autumn season (III). No significant effect of location in Zn concentration detected, but no study available regarding with our finding.

No significant effect of location in Mn concentration finding, and no study available regarding the finding. The low level of Mn in serum blood of grazing ewes in southern Jordan, and may the low level of Mn in soil and feed reflect the Mn level in serum (McDowell, 1981).

Cobalt concentration in blood serum not detected, there blow detection limits. Our finding agree with Pastrana and other (1991), they reported in study of sheep, were deficient during dry season, but disagree with Abdelrahman (2003) who found an adequate Co level in serum sheep in northern part of Jordan throughout the year.

In season III, no significant differ between of location on Cu, Zn and Mn concentration in blood serum of grazing ewes in southern Jordan. In general Cu concentration was adequate in the three locations, but marginal in Zn, deficient in Mn and Co.

4.2.3.2. Enzymes activity:

Table 18. Shows the effect of location on the activity of Gpx, GR, GST and CAT enzymes in whole blood of Awassi ewes in season III.

No significant difference (P>0.01) was found between Aiy and Al-Qaser, but Gpx activity in Al-Jedydeh was significantly differ (P<0.01) when compared with other locations. Moreover, numerically the Gpx activity in Aiy high (18.24 U/ g Hb) than Al-Jedydeh and Al-Qaser (12.04 and 16.41 U/g Hb, respectively) was detected.

A significant difference (P<0.001) in the activity of GR enzyme in whole blood of Awassi ewes within different locations was detected. The GR activity in Al-Jedydeh was higher (2890.03 U/g Hb) and significantly differ (P<0.001) than Aiy and Al-Qaser (1801.9 and 2085.2 U/g Hb, respectively).

A significant effect (P<0. 001) in the GST activity in whole blood of Awassi ewes was detected. The GST activity in Al-Qaser was significantly differs (P<0.001) than other locations. No significant difference (P>0.05) was detected in Aiy and Al-Jedydeh. Moreover, GST activity in blood of Awassi ewes in Al-Qaser was higher (2.04 U/g Hb) compare with Aiy and Al-Qaser (1.83 and 1.93 U/ g Hb, respectively).

No significant difference (P>0.05) was detected in activity of CAT in whole blood of Awassi ewes. Moreover, numerically high activity of CAT was found in Al-Qaser (2.47 U/ g Hb) compare within Aiy and Al-Jedydeh (2.05 and 1.81 U/g Hb, respectively).

The variation between locations in antioxidant enzyme activity might be due to the differences in feed available and level of the trace elements during season III, which considered as cofactor for these enzymes.

In season III, high activity of Gpx and CAT in Aiy and Al- Qaser were detected, and the highest activities of GR and GST were found in Al- Jedydeh. A significant difference between location on activities of

Gpx, GR and GST were observed, but no significant differences in activity of CAT.

4.2.4. Effect of location during season IV:

4.2.4.1. Trace elements:

The effect of locations in the concentrations of Copper (Cu), Zinc (Zn), Manganese (Mn), and Cobalt (Co) in the serum of the Awassi ewes in season IV reported in table 19. There were no significant differences (P>0.05) in Cu concentration in blood serum of grazing Awassi ewes. Numerically, Cu concentration in Al-Jedydeh (0.97 µg/ml) higher compared with Aiy and Al-Qaser (0.85 and 0.84 µg/ml, respectively). Moreover, the concentration of Cu fallen within adequate level in all locations.

No significant differences (P>0.05) in Zn concentration in blood serum of Awassi ewes were detected. Numerically, Zn concentration in Al-Jedydeh location (0.66 µg/ml) was higher compared within Aiy and Al-Qaser (0.53 and 0.62 µg/ml, respectively). Moreover, the concentration of Zn was fallen within the marginal level in all locations.

Manganese concentration in blood serum of grazing Awassi ewes were significantly differ (P<0.01) by location. Manganese concentration in Al-Jedydeh was significantly differs (P<0.01) than in Aiy and Al-Qaser, but no differences (P>0.05) reported in Aiy and Al-Qaser. Moreover, the values were deficient in all locations, the highest value were detected in Al-Qaser (0.21 µg/ml).

The concentrations of Co in blood serum of ewes were below detection limit, which indicated deficiency in three locations.

Most authors determined that the lowest serum Cu in the winter in cattle (Yokus *et al.*, 2004), this result disagree with our finding. Lack in available feedstuffs for grazing animal in these locations, might affect the level of Cu in serum blood. Abdelrahman (2003) reported that Cu concentration in blood serum of grazing sheep were in adequate level in northern part of Jordan.

This result agreed with White *et al.*, (1995) who reported a below adequate level during winter season. On the other hand, Abdelrahman (2003) reported low Zn level in blood serum of grazing sheep in northern Jordan throughout year. Lack of feed available may affect the level of Zn in blood serum of Awassi ewes in three locations in winter season (IV). No significant effect of location in Zn concentration in blood serum of grazing ewes.

Abdelrahman (2003) reported a deficiency on Mn concentration in blood serum of grazing ewes during winter season, our result supported the finding. Albel and other (1979) reported deficiency in Mn in liver sheep in winter season, were a deficiency occurred when low Mn level in available feed and soil may effect Mn level in animal (McDowell, 1981).

In season IV, no significant differences between location in Cu and Zn concentrations in blood serum of grazing ewes. Copper concentration were adequate and Zn were deficient, a significant concentration of Zn in Al-Qaser compared with other locations.

4.2.4.2. Enzymes activity:

Table 20. Shows the effect of location on the activities of Gpx, GR, GST and CAT enzymes in whole blood of Awassi ewes in season IV.

No significant difference (P>0.05) in the activity of Gpx enzyme in whole blood of Awassi ewes from location to another was detected, but numerically the high activity reported in Al-Jedydeh location (23.01 U/ g Hb). The lowest activity of Gpx in Aiy location (16.84 U/g Hb) was detected.

No significant difference (P>0.05) in the activity of GR enzyme from location to another was observed, but numerically low activity reported in Al-Qaser (1022.1 mU/ g Hb). The high activity of GR in Al-Jedydeh and Aiy (1110.92 and 1394.8 mU/ g Hb, respectively) was detected.

Significant effect (p<0.001) in the GST activity in whole blood of Awassi ewes was observed, the GST activity in Aiy was a significantly differ (P<0.001) than other locations. Moreover, GST activity in blood of ewes in Al-Qaser was highest (1.15 U/g Hb) and significantly differ (P<0.001) from the activity in Aiy and Al-Jedydeh (0.62 and 0.88 U/g Hb, respectively).

A significant difference (P<0.01) was detected in activity of CAT in whole blood of Awassi ewes within locations was detected. The CAT activity in Aiy location was the highest (2.83 U/g Hb) and significantly differ (P<0.01) from Al-Jedydeh and Al-Qaser (1.73 and 2.38 U/g Hb, respectively), but no significant difference (P>0.05) between Al-Jedydeh and Al-Qaser was detected.

The variations in Gpx, GR, CAT and GSTs activities during this season would be associated with location differences, since the available trace element during this season for animal is limited and mostly depend on the supplementation with feeding. Moreover, the lack of forage may be mostly the reason behinds the variations in trace elements concentrations during the winter.

In season IV, high activity of Gpx and GST in AL-Jedydeh and Al-Qaser were detected, and the highest activities of GR and CAT were found in Aiy. A significant difference between location on activities of GST and CAT were observed, but no significant differences in activities of Gpx And GR.

4.3. Correlations between trace element concentrations and enzyme activities:

Trace elements may interact with other minerals, nutrients or with non-nutrient (Lucille *et al.*, 1983). This interaction can occur in the diet, digestive system, or in tissues and cells. The interaction may be antagonistic or direct interaction during synthesis of structural protein, competition between elements for the active sites on an enzyme, or activation of some enzyme systems. Mineral antagonisms in tissues occur often by direct interaction between ions at an active sites, or competition for a common transport site or ligand (Hill, 1976).

Table 21. shows the correlations between the concentrations of Cu, Zn, Mn, and Co in the serum and activities of Gpx, GR, GST and CAT enzymes in whole blood of Awassi ewes throughout the year. There were negative significant correlation ($r=-0.33$; $P<0.05$) between Zn and Mn and enzymes were detected.

A negative significant correlation ($r=- 0.24$; $P<0.05$) of Zn with Mn and GR were detected, but no significant correlations of Zn with other minerals and enzymes. A positive significant correlation ($r= 0.44$; $P<0. 001$) of Mn with GR and GST activity was detected. On the other hand no significant correlation ($r= 0.02$; $P>0.05$) of Co with trace elements and enzymes were detected. A positive significant correlation ($r= 0.24$; $P<0.05$) of Gpx with CAT was found, but no significant correlation with others enzymes and minerals. A positive correlation ($r= 0.55$; $P<0. 001$) between GR and GST were detected, but no significant correlations with others were detected.

The trace elements Fe, Cu, Zn and Se are essential components of major antioxidant enzymes in blood such as CAT, Cu/Zn-SOD, Gpx, GR and GSTs. Since the status of trace elements levels in plasma and erythrocyte have traditionally been used to assess (L'abbe *et al.*, 1992; Tyrala *et al.*, 1996) their susceptible to direct oxidative damage and are convenient for the evaluation of cellular antioxidant systems such as CAT, GR, GST and Gpx activities in erythrocytes (Ceballos *et al.*, 1992; Glauser *et al.*, 1999).

A correlation analysis between the activity values of enzyme and the content of essential and risk elements was done. We found a correlation dependent (P<0.05) between Gpx activity value and Cu content (r= 0.09). Moreover, a negative correlation between the CAT and GR and GST activity and the Cu content (r=-0.04, -0.02 and -0.04, respectively). The significant correlation (r = 0.24; P< 0.04) between Gpx and catalase activity values is also in agreement with the study by Oddo *et al*., (1999). The antioxidant enzymes are dependently associated with trace elements level.

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Appendix
(I)

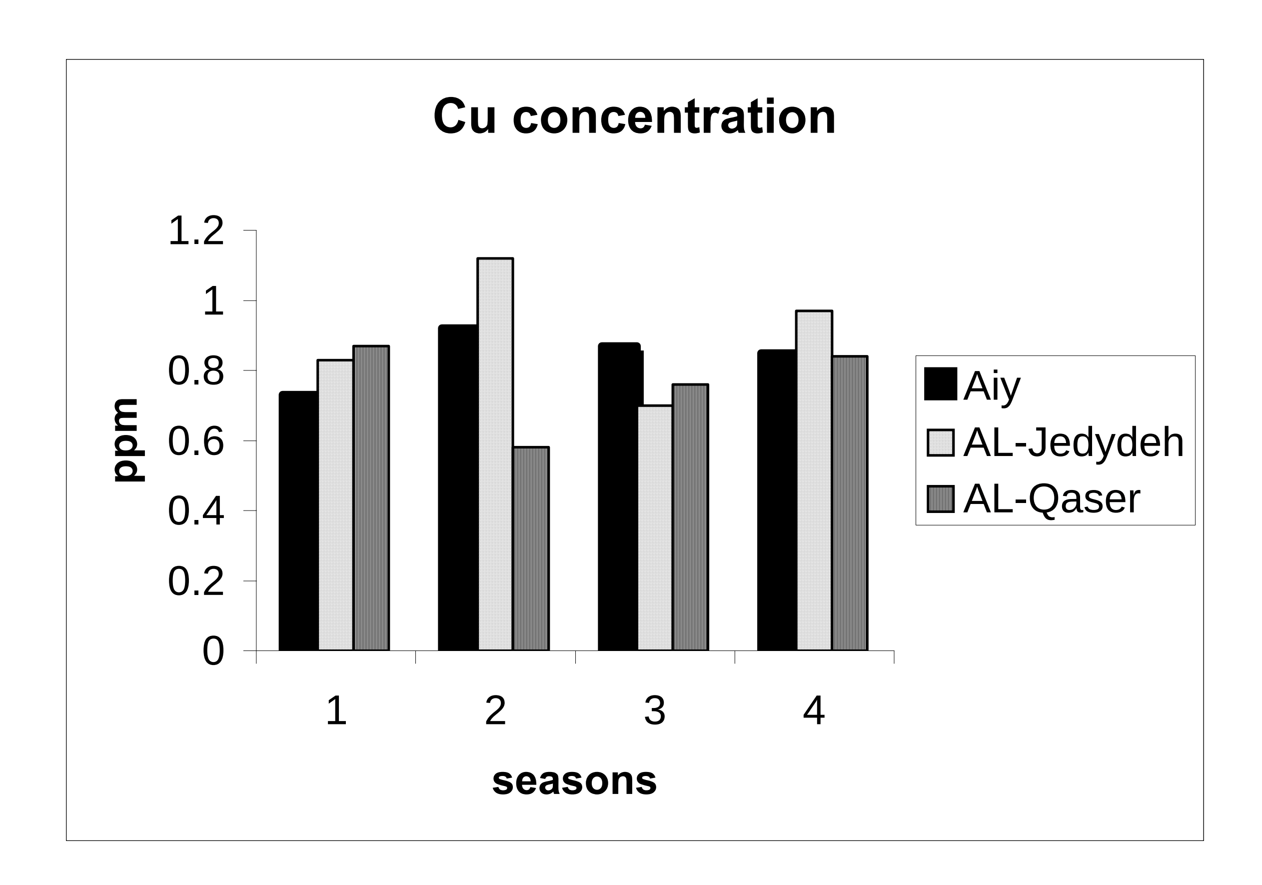


Figure 1.

Concentrations of Cu in serum of grazing Awassi sheep throughout the year in Aiy, Al-Jedydeh and Al-Qaser

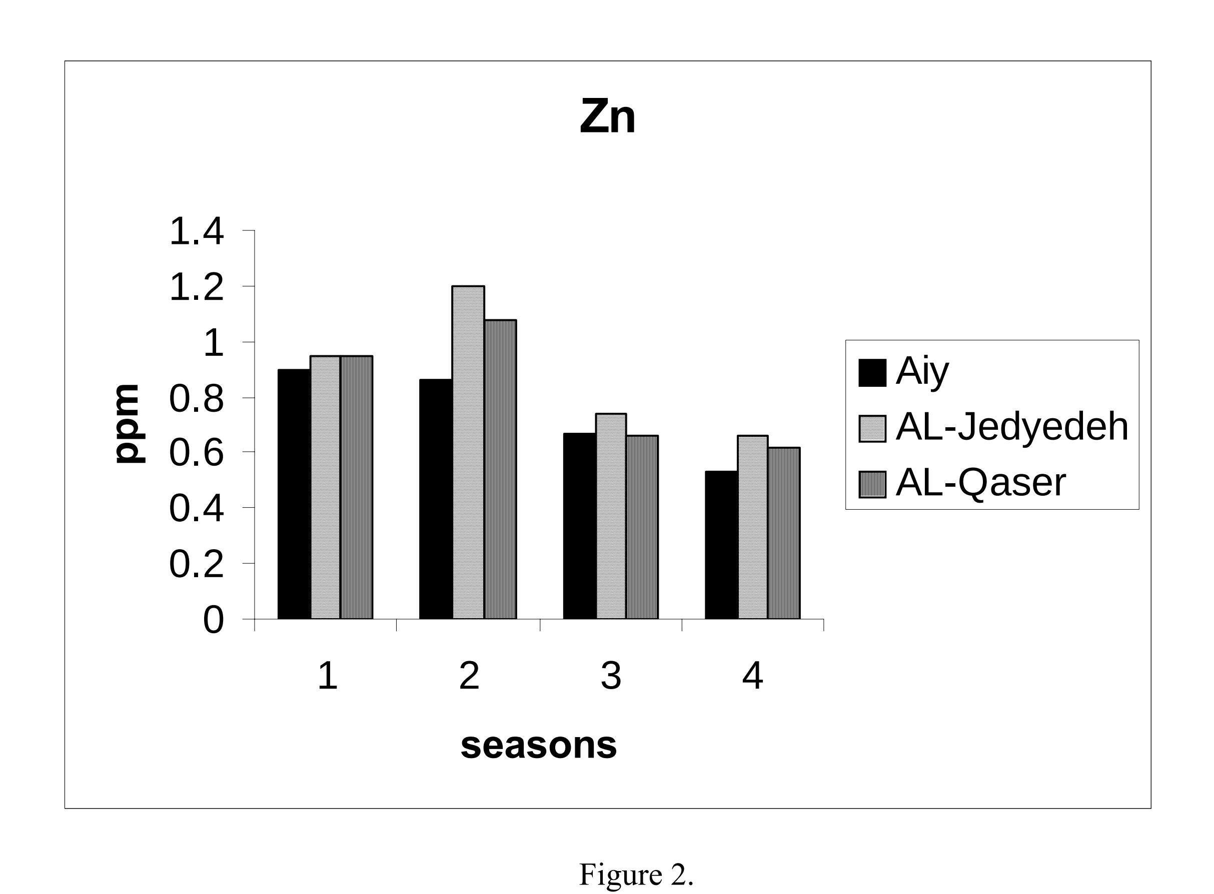


Figure 2.

Concentrations of Zn in serum of grazing Awassi sheep throughout the year in Aiy, Al-Jedydeh and Al-Qaser

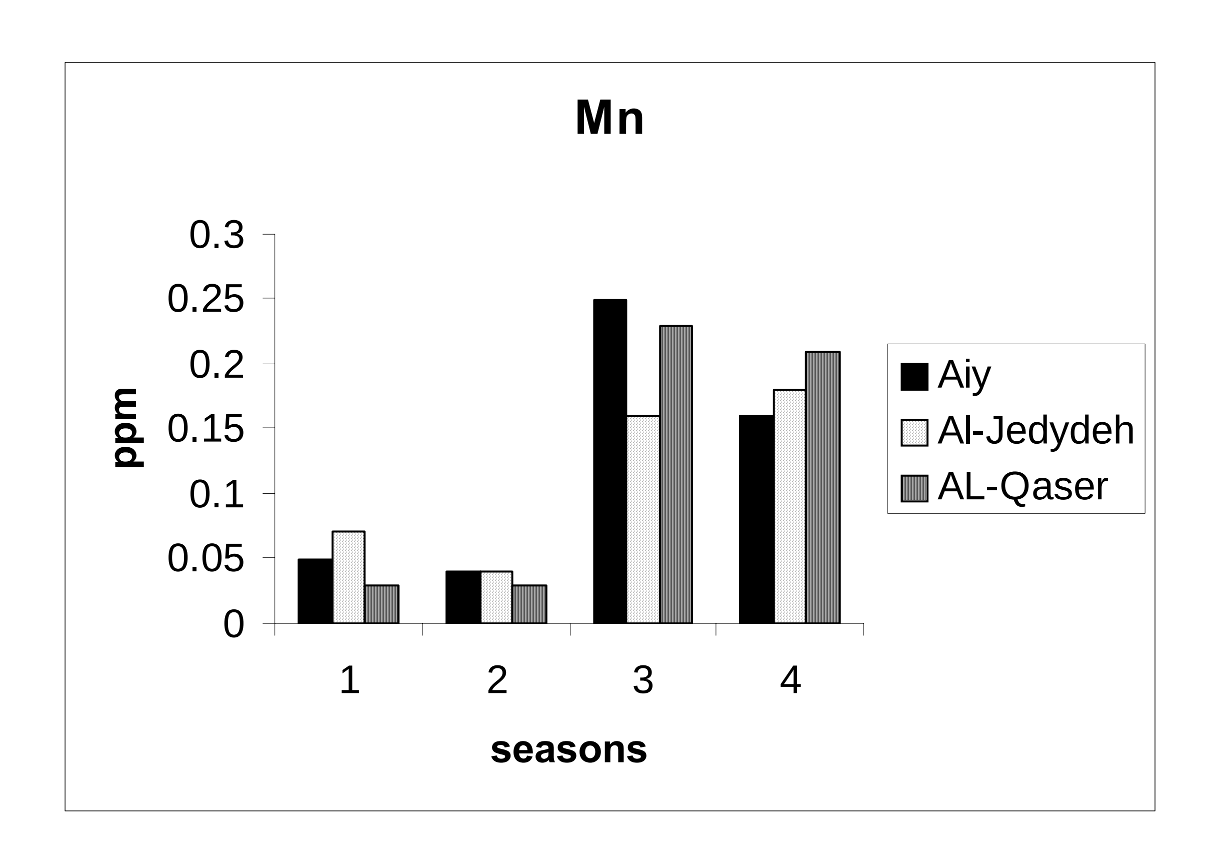


Figure 3.

Concentrations of Mn in serum of grazing Awassi sheep throughout the year in Aiy, Al-Jedydeh and Al-Qaser

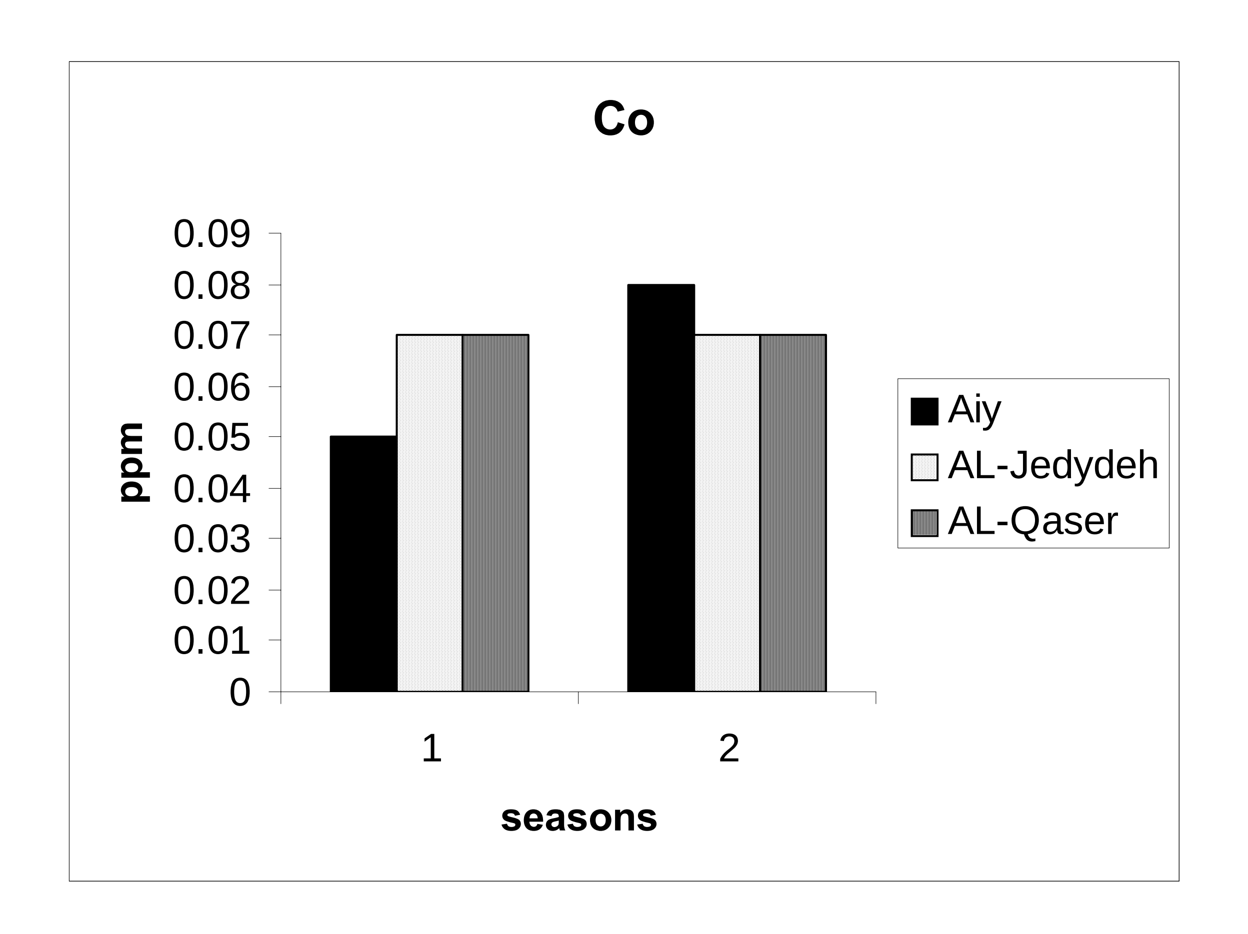


Figure 4.

Concentrations of Co in serum of grazing Awassi sheep throughout the year in Aiy, Al-Jedydeh and Al-Qaser

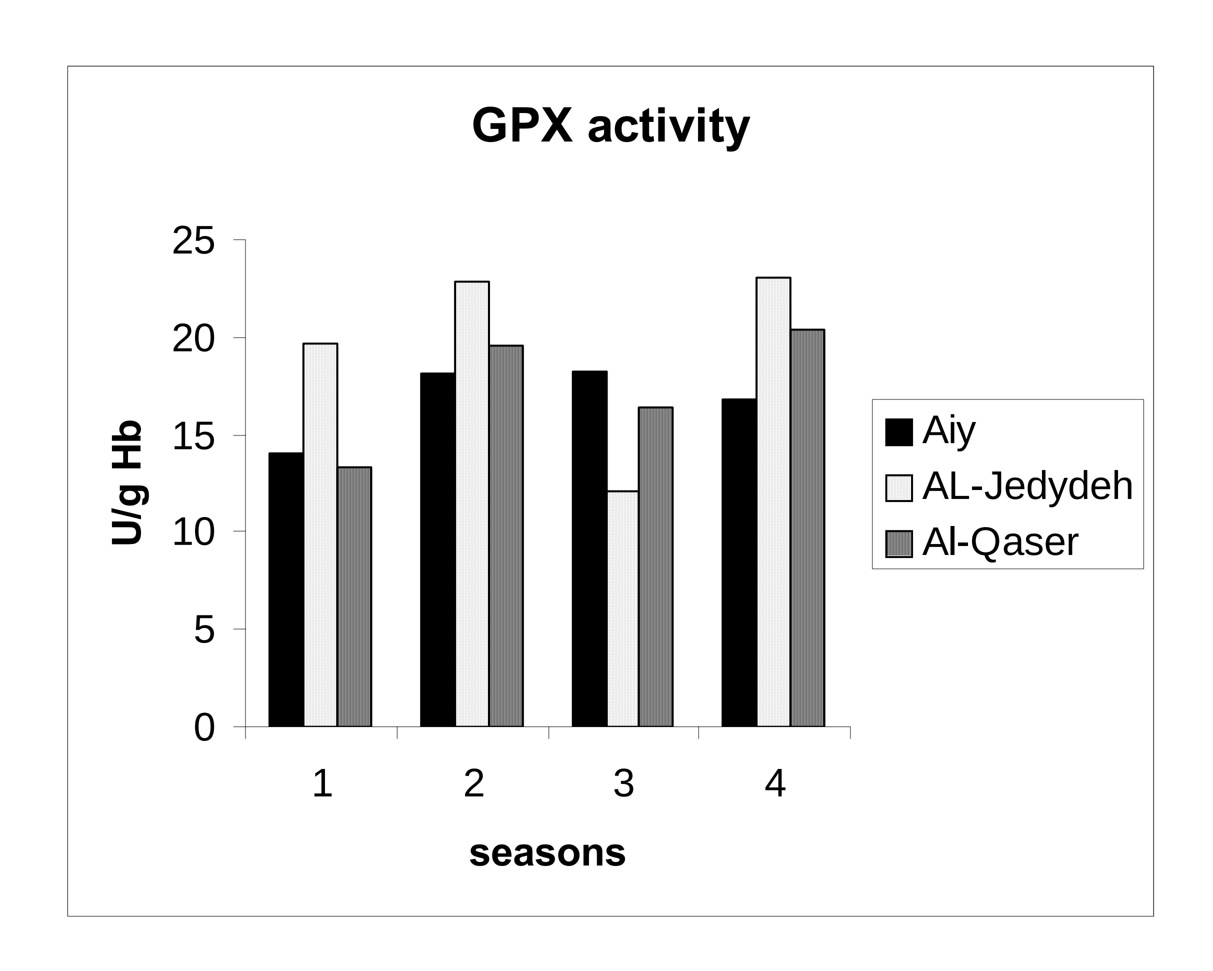


Figure 5.

Activities of Gpx enzyme in whole blood of grazing Awassi sheep throughout the year in Aiy, Al-Jedydeh and Al-Qaser

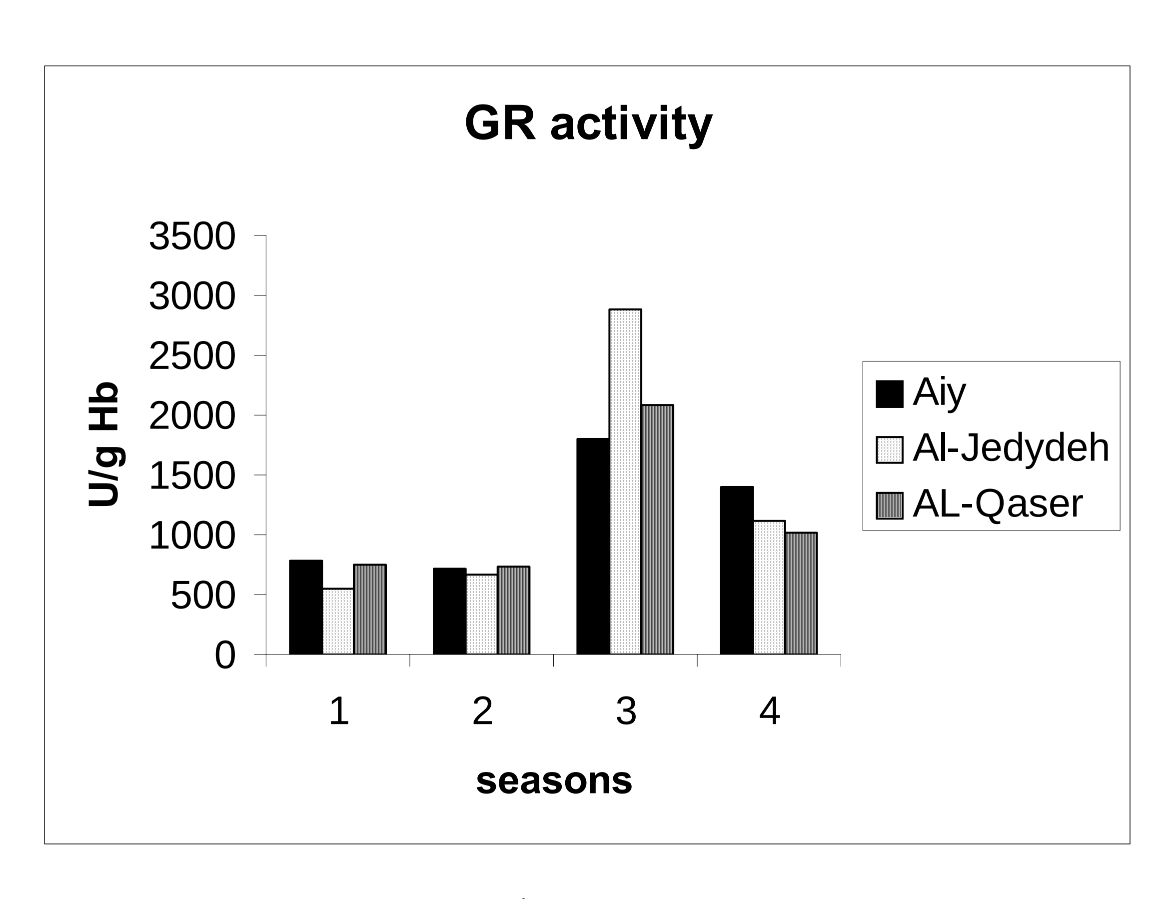


Figure 6.

Activities of GR enzyme in whole blood of grazing Awassi sheep throughout the year in Aiy, Al-Jedydeh and Al-Qaser

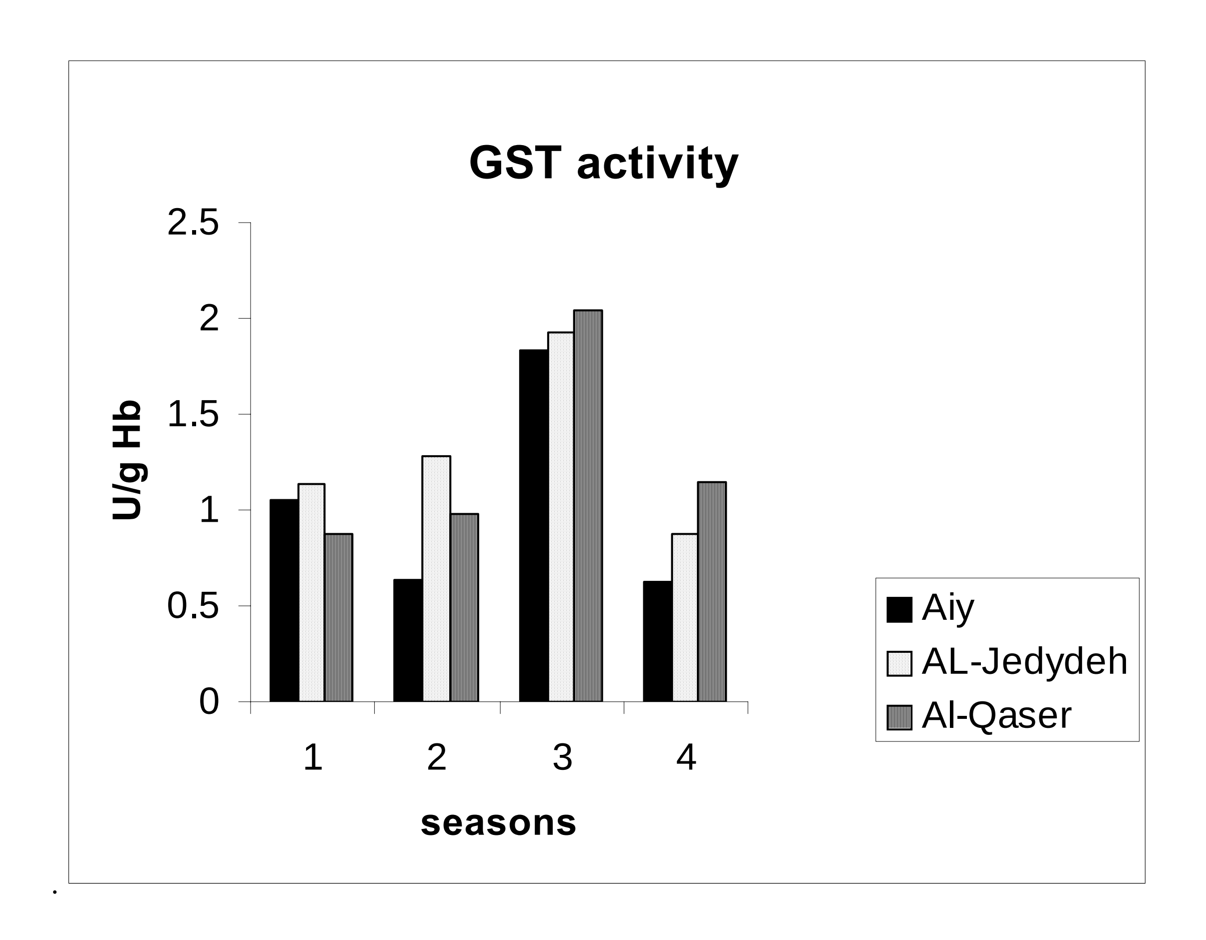


Figure 7.

Activities of GST enzyme in whole blood of grazing Awassi sheep throughout the year in Aiy, Al-Jedydeh and Al-Qaser

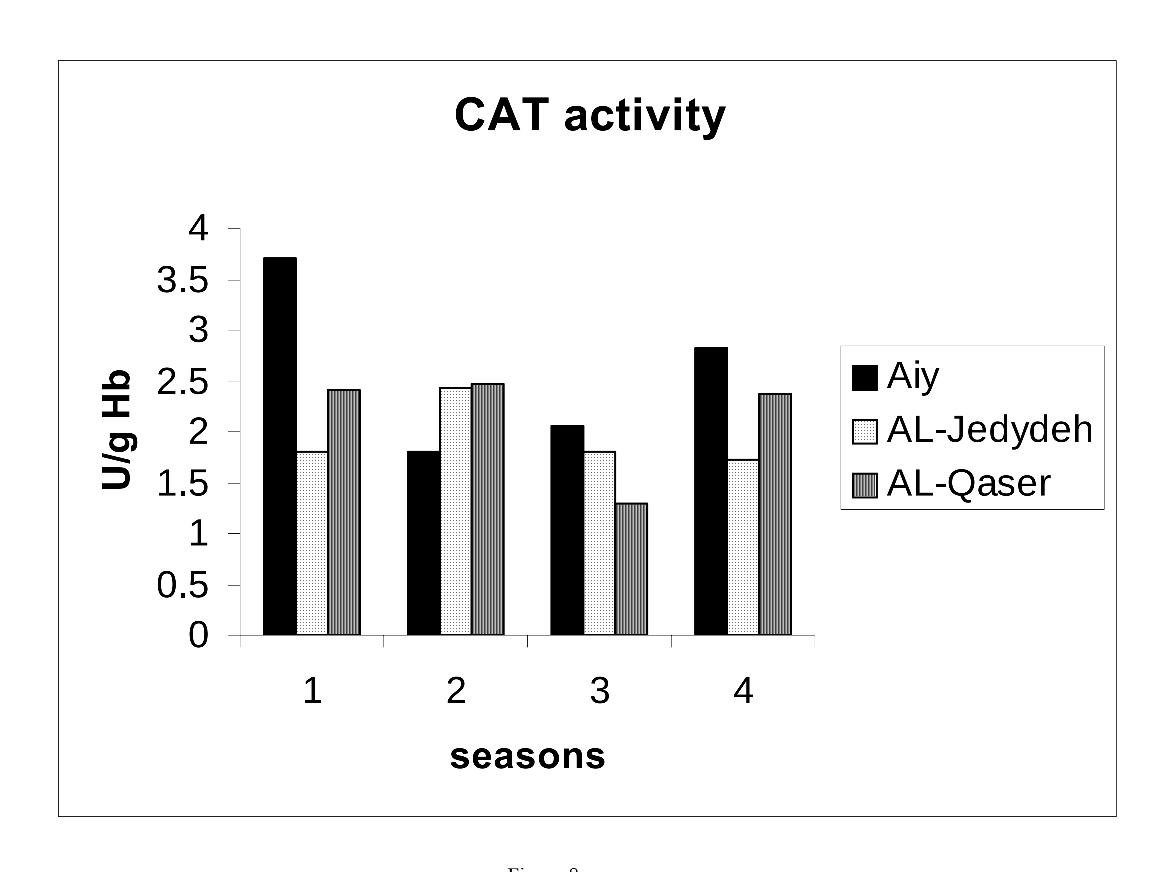


Figure 8.

Activities of CAT enzyme in whole blood of grazing Awassi sheep throughout the year in Aiy, Al-Jedydeh and Al-Qaser

Appendix (II)

1. Instruments:

1. Spectronic GENESYS 2(Milton Roy company, U.S.A.)
2. Atomic Absorption Flame and Hydride generator (Varian. GTA 100 Spectra AA- 200).
3. Gs-6 Centrifuge. (Beckman, U.S.A).
4. Laboratory centrifuge GmbH. (sigma, Germany).
5. Microprocessor PH meter. (WTW, Germany).
6. Electronic pipetting center. (HTL, High tech lab).
7. Balance. (OHAUS, U.S.A.).

2. Equipments:

1. Vaccutte tubes (hebraized and non- hebraized).
2. K_2HPO_4 and $KH_2PO_{4.}$, (Sigma- Aldrich chemi).
3. Trichloroaceticacid (1 kg). (Janssen chimica).
4. Hydrogen peroxide 30% (H_2O_2). (GAINLAND Chemical company, U.K.)
5. 1-chloro-2, 4-dinitrobenzene ($C_3H_3CIN_2O_4$) (MERCK-Schuchardt, Germany).
6. Tris- (hydroxymethyl)-amino methane (1 kg). (Reidel- de Haen AG, Germany).
7. Sodium Chloride (NaCl) (5 kg). (Sigma-Aldrich company, Germany).
8. Ethylene diamine tetra acetic acid (EDITA) disodium salt. LR. ($C_{10}H_{12}N_4Na_2O_8.2H_2O$). (S.D. Fine-chem. Ltd)
9. Hydrochloric acid (HCl) (SURECHEM).
10. Absolute Alcohol A.R. (Ethyl Alcohol C_2H_5OH). (HAYMAN, England.
11. Glutathione. (25 gm). Reduced form Minimum 98% (Sigma- Aldrich, Germany).
12. L-Glutathione oxidized (5gm). Min 98% (Applichem, Germany).
13. Glutathione Reductase. From wheat germ (1 gm). (Sigma- Aldrich, Germany).
14. NADPH-Tetra salt (500 mg). (Applichem, Germany).
15. Hemoglobine Reagent Set (6×1L Hemoglobin reagent), (1×30 ml Hemoglobin standard). (Techo Diagnostics, U.S.A.).

Chapter V
Summary and Recommendations

5.1. Summary

From this data presented in southern Jordan, it is possible to conclude that some trace elements and enzymes activity are significantly differ by seasons and Location, as show below.

5.1.1 Effect of season

1. **In Aiy, a Cu concentration in the blood serum of Awassi ewes is adequate throughout the year. Zinc is adequate only during season I (spring) and II (summer), but marginal during the other seasons. Moreover, Mn and Co were deficient throughout the year. In addition, high activity of Gpx during season II (summer) and III (autumn), GR during season III (autumn) but low in other seasons, high activities of GST and CAT during season I (spring) and III (autumn).**
2. In Al- Jedydeh, Cu concentration is adequate during season I (spring), II (summer) and IV (winter), but marginal during season III (autumn). Zinc concentration was marginal during seasons III (autumn) and IV (winter), but Co is deficiency throughout the year. In addition, high activity of Gpx during season II (summer) and IV(winter), but low in other seasons. High activity for GR and GST during season III (autumn) and high activity of CAT during seasons II (summer) and III (autumn).
3. **In Al-Qaser, Copper deficiency is during season II (summer), Zn during season III (autumn) and IV (winter). Mn and Co deficient throughout the year. In addition, high activity of Gpx during season II (summer) and IV (winter). High activity of GR and GST during season III (autumn) and high activity of CAT during seasons I (spring) and IV (winter).**

5.1.2 Effect of location

1. **In season I (spring), Cu concentration is adequate in Al-Jedydeh and Al-Qaser, but marginal in Aiy. Zinc concentration is adequate in season I. Moreover, Mn and Co is deficient in all location. In addition, high activity of Gpx and GST in Al-Jedydeh. High activities of GR and CAT in Aiy and Al- Qaser.**
2. **In season II (summer), Cu concentration is adequate in Aiy and Al-Jedydeh, but marginal in Al-Qaser. Zinc concentration is adequate in three locations. Manganese and Co were deficient in three location. In addition, a high activity of Gpx and GST in Al-Jedydeh, high activity of GR and CAT in Al-Jedydeh and Al- Qaser.**
3. **In season III (autumn), in general Cu concentration is in adequate in the three locations, but marginal in Zn, deficient in Mn and Co. In addition, a high activity of Gpx and CAT in Aiy and Al- Qaser, high activity of GR and GST in Al- Jedydeh.**
4. **In season IV (winter), Cu concentration is adequate, but Zn, Mn and Co is deficient. In addition, a high activity of Gpx and GST in Al-Jedydeh and Al- Qaser, high activity of GR and CAT in Aiy.**

5.1.3. Correlations

1. **There are negative correlation (P<0.05) between Cu and Mn, a significant negative correlation (P<0.05) of Zn with Mn and GR activities, and a significant positive correlation (P<0.001) of Mn with GR and GST activities.**
2. **A significant positive correlation (P<0.05) of Gpx with CAT.**
3. **A significant positive correlations (P<0.001) between GR and GST.**

5.2. Recommendations

1. Concentration of other essential minerals and enzyme activities such as Se level and SOD activities in tissues must be studied to have complete view of status of grazing sheep in southern Jordan.

2. Concentrations of minerals and enzymes activities in other tissues such as liver, kidney, bone and other organs, as well as milk and meat should be studied.

3. An economically minerals supplementations program adapted to local conditions is very crucial to overcomes deficiencies and improve sheep health, productivity and consequently human health.

4. The rehabilitation of grazing resources (range) should be arranged to achieve wide range of feed capability.

Results

Table 1.

Trace elements concentrations in feed of grazing Awassi sheep in Aiy throughout the year

	Cu	Zn	Mn	Co
Season I[1]				
Barley	5.08	48.15	ND	ND
Whole barley	7.40	13.33	ND	ND
Pasture	3.98	217.78	ND	ND
Season II[2]				
Barley	3.05	18.35	ND	ND
Wheat	.78	15.85	ND	ND

Straw				
Season III[3]				
Whole Barley	9.13	43.75	1.18	ND
Pasture	2.03	10.13	ND	ND
Wheat straw	0.4	7.3	0.7	ND
Season IV[4]				
Wheat Straw	0.7	15.0	ND	ND
Barley	2.50	16.0	1.0	ND
Pasture	3.40	31.95	0.9	ND

Diet= ppm of Dry matter.
[1]Season I (Spring): from April to May.
[2]Season II (Summer): from June to September.
[3]Season III (Autumn): from October to December.
[4]Season IV (Winter): from January to March.
ND= not detected.

Table 2.
Trace elements concentrations in feed of grazing Awassi ewes in Al-Jedydeh throughout the year

	Cu	Zn	Mn	Co
Season I[1]				
Barley	6.0	30.0	ND	ND
Wheat Straw	0.50	7.55	ND	ND
Wheat bran	7.15	65.73	ND	ND
Season II[2]				
Whole Barley	2.40	31.03	ND	ND
Wheat straw	0.6	8.0	ND	ND

Season III[3]				
Barley + Wheat bran	5.50	38.73	1.20	ND
Season IV[4]				
Wheat Straw	0.4	7.3	0.7	ND
Wheat Straw + Barley	5.43	28.25	.43	ND
Pasture	9.25	36.98	0.88	ND

Diet= ppm Dry matter.
[1]Season I (Spring): from April to May.
[2]Season II (Summer): from June to September.
[3]Season III (Autumn): from October to December.
[4]Season IV (Winter): from January to March.
ND= not detected.

Table 3.
Trace elements concentrations in feed of grazing Awassi ewes in Al-Qaser throughout the year

	Cu	Zn	Mn	Co
Season I[1]				
Barley	4.50	32.0	ND	ND
Wheat Straw	4.50	8.0	ND	ND
Pasture	4.08	198.0	ND	ND
Season II[2]				
Barley	3.50	40.50	ND	ND
Wheat	6.80	76.85	ND	ND

Bran				
Pasture	6.0	36.05	ND	ND
Season III[3]				
Barley	6.0	40.33	0.85	ND
Wheat bran	5.0	36.70	1.10	ND
Season IV[4]				
Barley	7.0	39.0	0.90	ND
Pasture	8.13	41.08	1.18	ND

Diet= ppm Dry matter.
[1]Season I (Spring): from April to May.
[2]Season II (Summer): from June to September.
[3]Season III (Autumn): from October to December.
[4]Season IV (Winter): from January to March.
ND= not detected.

Table 4.
The concentration of trace elements in soil throughout the year in Aiy

	Seasons			
	I[1]	II[2]	III[3]	IV[4]
Cu, ppm	0.77	0.87	0.81	1.51
Zn, ppm	5.00	4.29	4.00	15.53
Mn, ppm	4.86	4.29	6.25	9.25
Co, ppm	0.14	0.15	0.1	0.35

[1]Season I (Spring): from April to May.
[2]Season II (Summer): from June to September.
[3]Season III (Autumn): from October to December.
[4]Season IV (Winter): from January to March.

Table 5.
The concentration of trace elements in soil throughout the year in Al-Jedydeh

	Seasons			
	I[1]	II[2]	III[3]	IV[4]
Cu, ppm	0.85	0.89	0.81	0.70
Zn, ppm	2.72	1.81	1.4	14.75
Mn, ppm	10.01	6.81	10.25	18.50
Co, ppm	0.17	0.15	0.09	0.16

[1]Season I (Spring): from April to May.
[2]Season II (Summer): from June to September.
[3]Season III (Autumn): from October to December.
[4]Season IV (Winter): from January to March.

Table 6.
The concentration of trace elements in soil throughout the year in Al-Qaser

	Seasons			
	I[1]	II[2]	III[3]	IV[4]
Cu, ppm	0.82	1.18	.81	0.79
Zn, ppm	6.41	10.20	5.00	7.50
Mn, ppm	4.41	7.60	8.28	9.08

Co, ppm	0.12	0.32	0.11	0.15

[1]Season I (Spring): from April to May.
[2]Season II (Summer): from June to September.
[3]Season III (Autumn): from October to December.
[4]Season IV (Winter): from January to March.

Table 7.
Effect of seasons on the concentration of trace elements in serum of grazing Awassi ewes throughout the year in Aiy

	Season					
	I[1]	II[2]	III[3]	IV[4]	SE	Sig.
Cu, μg/ml	0.73	0.92	0.87	0.85	0.09	NS
Zn, μg/ml	0.90[a]	0.86[ac]	0.67[b]	0.53[b]	0.08	***
Mn, μg/ml	0.05[a]	0.04[a]	0.25[b]	0.16[c]	0.04	***
Co, μg/ml	0.05[a]	0.08[b]	ND	ND	-	*

[1]Season I (Spring): from April to May.
[2]Season II (Summer): from June to September.
[3]Season III (Autumn): from October to December.
[4]Season IV (Winter): from January to March.
[abc] Mean values with different superscript within row are significantly different.
*, **, ***, Significantly different at P<0.05, P<0.01, P<0.001, respectively.
NS= not significantly different (P>0.05).
ND= Not Detected.
SE= Standard errors of means.

Table 8.
Effect of seasons on the level of antioxidant enzymes activity in whole blood in grazing Awassi ewes throughout the year in Aiy

	Season					
	I[1]	II[2]	III[3]	IV[4]	SE	Sig.
Gpx, U/g Hb	14.05	18.14	18.24	16.84	2.10	NS
GR, ml U/g Hb	775.5^{a}	724.4^{a}	1801.9^{b}	1394.8^{ab}	316.03	**
GST, U/g Hb	1.05^{a}	$.64^{b}$	1.83^{c}	0.62^{b}	0.11	***

CAT, U/g Hb	3.70[a]	1.8[b]	2.05[b]	2.83[ab]	0.41	***

[1]Season I (Spring): from April to May.
[2]Season II (Summer): from June to September.
[3]Season III (Autumn): from October to December.
[4]Season IV (Winter): from January to March.
[abc] Mean values with different superscript within row are significantly different.
*, **, ***, Significantly different at P<0.05, P<0.01, P<0.001, respectively.
NS= not significantly different (P>0.05).
SE= Standard errors of means.

Table 9.
Effect of seasons on the concentration of trace elements in serum of grazing Awassi ewes throughout the year in Al-Jedydeh

	Season					
	I[1]	II[2]	III[3]	IV[4]	SE	Sig.
Cu, μg/ml	0.83[a]	1.12[ab]	0.70[ac]	0.97[a]	0.12	**
Zn, μg/ml	0.95[a]	1.20[ac]	0.74[ab]	0.66[b]	0.09	***
Mn, μg/ml	0.07[a]	0.04[b]	0.16[c]	0.18[c]	0.04	***

Co, µg/ml	0.07	0.07	ND	ND	-	NS

[1]Season I (Spring): from April to May.
[2]Season II (Summer): from June to September.
[3]Season III (Autumn): from October to December.
[4]Season IV (Winter): from January to March.
[abc] Mean values with different superscript within row are significantly different.
*, **, ***, Significantly different at P<0.05, P<0.01, P<0.001, respectively.
NS= not significantly different (P>0.05).
ND= Not Detected.
SE= Standard errors of means.

Table 10.
Effect of seasons on the level of antioxidant enzymes activity in whole blood in grazing Awassi ewes throughout the year in Al-Jedydeh

	Season					
	I[1]	II[2]	III[3]	IV[4]	SE	Sig.
Gpx, U/g Hb	19.70[a]	22.80[a]	12.04[ac]	23.01[ab]	3.80	*
GR, ml U/g Hb	542.83[a]	660.0[ac]	2890.03[b]	1110.92[a]	380.12	***
GST, U/g Hb	1.14[a]	1.28[ab]	1.93[b]	0.88[a]	0.26	**

CAT, U/g Hb	1.80	2.44	1.81	1.73	0.45	NS

[1]Season I (Spring): from April to May.
[2]Season II (Summer): from June to September.
[3]Season III (Autumn): from October to December.
[4]Season IV (Winter): from January to March.
[abc] Mean values with different superscript within row are significantly different.
*, **, ***, Significantly different at P<0.05, P<0.01, P<0.001, respectively.
NS= not significantly different (P>0.05).
SE= Standard errors of means.

Table 11.
Effect of seasons on the concentration of trace elements in serum of grazing Awassi ewes throughout the year in Al-Qaser

	Season					
	I[1]	II[2]	III[3]	IV[4]	SE	Sig.
Cu, μg/ml	0.87[a]	0.58[b]	0.76[a]	0.84[ac]	0.09	**
Zn, μg/ml	0.95[a]	1.08[a]	0.66[b]	0.62[b]	0.09	***
Mn, μg/ml	0.03[a]	0.03[a]	0.23[b]	0.21[b]	0.01	***

Co, μg/ml	0.15	0.14	ND	ND	-	NS

[1]Season I (Spring): from April to May.
[2]Season II (Summer): from June to September.
[3]Season III (Autumn): from October to December.
[4]Season IV (Winter): from January to March.
[abc] Mean values with different superscript within row are significantly different.
*, **, ***, Significantly different at P<0.05, P<0.01, P<0.001, respectively.
NS= not significantly different (P>0.05).
ND= Not Detected.
SE= Standard errors of means.

Table 12.
Effect of seasons on the level of antioxidant enzymes activity in whole blood in grazing Awassi ewes throughout the year in Al-Qaser

	Season					
	I[1]	II[2]	III[3]	IV[4]	SE	Sig.
Gpx, U/g Hb	13.30[a]	19.60[b]	16.41[a]	20.39[b]	2.58	*
GR, ml U/g Hb	752.97[a]	734.7[a]	2085.2[b]	1022.1[a]	220.75	***
GST, U/g Hb	0.88[a]	0.98[a]	2.04[b]	1.15[a]	0.14	**

CAT, U/g Hb	2.41[a]	2.47[a]	1.29[b]	2.38[a]	0.31	**

[1]Season I (Spring): from April to May.
[2]Season II (Summer): from June to September.
[3]Season III (Autumn): from October to December.
[4]Season IV (Winter): from January to March.
[abc] Mean values with different superscript within row are significantly different.
*, **, ***, Significantly different at P<0.05, P<0.01, P<0.001, respectively.
NS= not significantly different (P>0.05).
ND= Not Detected.
SE= Standard errors of means.

Table 13.
Effect of locations on the concentration of trace elements in serum of grazing Awassi ewes throughout the year in Aiy, Al- Jedydeh and Al-Qaser in season I

	Locations				
	Aiy	Jedydeh	Qaser	SE	Sig.
Cu, µg/ml	0.73	0.83	0.87	0.07	NS
Zn, µg/ml	0.90	0.95	0.98	0.10	NS
Mn, µg/ml	0.05	0.07	0.03	0.03	NS

Co, μg/ml	0.05[a]	0.07[a]	0.15[b]	0.03	***

[abc] Mean values with different superscript within row are significantly different.
*, **, ***, Significantly different at P<0.05, P<0.01, P<0.001, respectively.
NS= not significantly different (P>0.05).
SE= Standard errors of means.

Table 14.
Effect of location on the activity of antioxidant enzymes activity in whole blood grazing Awassi ewes throughout the year in Aiy, Al-Jedydeh and Al-Qaser in season I

	Locations				
	Aiy	Jedydeh	Qaser	SE	Sig.
Gpx, U/g Hb	14.05	19.70	13.3	3.25	NS
GR, ml U/g Hb	775.5[a]	542.83[ab]	752.97[a]	133.20	***
GST, U/g Hb	1.05	1.14	0.88	0.15	NS

CAT, U/g Hb	3.70[a]	1.80[b]	2.41[b]	0.38	***

[abc] Mean values with different superscript within row are significantly different.
*, **, ***, Significantly different at P<0.05, P<0.01, P<0.001, respectively.
NS= not significantly different (P>0.05).
SE= Standard errors of means.

Table 15.
Effect of locations on the concentration of trace elements in serum of grazing Awassi ewes throughout the year in Aiy, Al- Jedydeh and Al-Qaser in season II

	Locations				
	Aiy	Jedydeh	Qaser	SE	Sig.
Cu, μg/ml	0.92[a]	1.12[a]	0.58[b]	0.09	***
Zn, μg/ml	0.86[a]	1.20[b]	1.08[a]	0.09	**
Mn, μg/ml	0.04[a]	0.04[a]	0.03[b]	0.06	*

Co, µg/ml	0.08[a]	0.07[a]	0.13[b]	0.01	***

[abc] Mean values with different superscript within row are significantly different.
*, **, ***, Significantly different at P<0.05, P<0.01, P<0.001, respectively.
NS= not significantly different (P>0.05).
SE= Standard errors of means.

Table 16.
Effect of location on the activity of antioxidant enzymes activity in whole blood grazing Awassi ewes throughout the year in Aiy, Al-Jedydeh and Al-Qaser in season II

		Locations			
	Aiy	Jedydeh	Qaser	SE	Sig.
Gpx, U/g Hb	18.14[a]	22.80[ab]	19.60[ac]	2.81	**
GR, ml U/g Hb	724.4	660.0	734.7	147.5	NS
GST, U/g Hb	0.64[a]	1.28[b]	0.98[a]	0.25	*

CAT, U/g Hb	1.80	2.44	2.47	0.37	NS

[abc] Mean values with different superscript within row are significantly different.
*, **, ***, Significantly different at P<0.05, P<0.01, P<0.001, respectively.
NS= not significantly different (P>0.05).
SE= Standard errors of means.

Table 17.
Effect of locations on the concentration of trace elements in serum of grazing Awassi ewes throughout the year in Aiy, Al- Jedydeh and Al-Qaser in season III

	Locations				
	Aiy	Jedydeh	Qaser	SE	Sig.
Cu, μg/ml	0.87	0.70	0.76	0.13	NS
Zn, μg/ml	0.67	0.74	0.66	0.09	NS
Mn,	0.25	0.16	0.23	0.09	NS

µg/ml					
Co, µg/ml	ND	ND	ND	-	-

[abc] Mean values with different superscript within row are significantly different.
*, **, ***, Significantly different at P<0.05, P<0.01, P<0.001, respectively.
NS= not significantly different (P>0.05).
SE= Standard errors of means.
ND= Not Detected.

Table 18.
Effect of location on the activity of antioxidant enzymes activity in whole blood grazing Awassi ewes throughout the year in Aiy, Al-Jedydeh and Al-Qaser in season III

	Locations				
	Aiy	Jedydeh	Qaser	SE	Sig.
Gpx, U/g Hb	18.24^a	12.04^b	16.41^a	2.30	**
GR, ml U/g Hb	1801.9^a	2890.03^b	2085.2^c	421.76	***
GST, U/g Hb	1.83^a	1.93^a	2.04^b	0.19	***

CAT, U/g Hb	2.05	1.81	2.47	0.35	NS

[abc] Mean values with different superscript within row are significantly different.
*, **, ***, Significantly different at P<0.05, P<0.01, P<0.001, respectively.
NS= not significantly different (P>0.05).
SE= Standard errors of means.

Table 19.
Effect of locations on the concentration of trace elements in serum of grazing Awassi ewes throughout the year in Aiy, Al- Jedydeh and Al-Qaser in season IV

	Locations				
	Aiy	Jedydeh	Qaser	SE	Sig.
Cu, µg/ml	0.85	0.97	0.84	0.10	NS
Zn, µg/ml	0.53	0.66	0.62	0.07	NS
Mn, µg/ml	0.16^a	0.18^a	0.21^b	0.02	**

Co, μg/ml	ND	ND	ND	-	-

[abc] Mean values with different superscript within row are significantly different.
*, **, ***, Significantly different at P<0.05, P<0.01, P<0.001, respectively.
NS= not significantly different (P>0.05).
SE= Standard errors of means.
ND= Not Detected.

Table 20.
Effect of location on the activity of antioxidant enzymes activity in whole blood grazing Awassi ewes throughout the year in Aiy, Al-Jedydeh and Al-Qaser in season IV

		Locations			
	Aiy	Jedydeh	Qaser	SE	Sig.
Gpx, U/g Hb	16.84	23.01	20.39	3.21	NS
GR, ml U/g Hb	1394.8	1110.92	1022.1	416.45	NS
GST, U/g Hb	0.62^{a}	0.88^{b}	1.15^{c}	0.12	***

CAT, U/g Hb	2.83[a]	1.73[b]	2.38[a]	0.46	**

[abc] Mean values with different superscript within row are significantly different.
*, **, ***, Significantly different at $P<0.05$, $P<0.01$, $P<0.001$, respectively.
NS= not significantly different ($P>0.05$).
SE= Standard errors of means.

Table 21.
Correlations between trace elements and enzymes activity in blood of grazing Awassi ewes in three locations throughout the year

	Cu	Zn	Mn	Co	Gpx	GR	GST	CAT
Cu	…							
Zn	0.18	…						
Mn	-0.05	-0.33*	…					
Co	-0.33*	0.32	0.04	…				
Gpx	0.09	0.07	0.19	-0.004	…			
GR	-0.02	-0.24*	0.43***	0.13	-0.09	…		

GST	-0.04	0.02	0.44***	-0.12	0.13	0.55***	…	
CAT	-.04	0.11	-0.17	0.02	0.24*	-0.08	-0.06	…

*, **, ***, Significantly different at P<0.05, P<0.01, P<0.001, respectively.
Total # of sample for:
(Cu, Zn, Gpx, GR, GST, CAT)= 96.
(Mn)= 80.
(Co)= 37.